PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 11 August 2000 (11.08.00)	
International application No. PCT/US99/31230	**Applicant's or agent's file reference** RDAG-120 PCT
International filing date (day/month/year) 29 December 1999 (29.12.99)	**Priority date** (day/month/year) 29 December 1998 (29.12.98)
Applicant BARHAM, Robert et al	

1. The designated Office is hereby notified of its election made:

   [X] in the demand filed with the International Preliminary Examining Authority on:

   02 June 2000 (02.06.00)

   [ ] in a notice effecting later election filed with the International Bureau on:

   _____

2. The election [X] was

   [ ] was not

   made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Nestor Santesso
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Form PCT/IB/331 (July 1992)

US9931230

PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

14

Applicant's or agent's file reference RDAG-120 PCT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US99/31230	International filing date *(day/month/year)* 29 DECEMBER 1999	Priority date *(day/month/year)* 29 DECEMBER 1998
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant R&D AG INC.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report

   II ☐ Priority

   III ☐ Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV ☐ Lack of unity of invention

   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI ☐ Certain documents cited

   VII ☐ Certain defects in the international application

   VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
02 JUNE 2001	23 MARCH 2001
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Dorothea Lawrence For MELISSA KIMBALL Telephone No. (703) 308-0196

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US99/31230

I. Basis of the report

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed

[X] the description:
pages 1-102 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of

[X] the claims:
pages 103-108 , as originally filed
pages NONE , as amended (together with any statement) under Article 19
pages NONE , filed with the demand
pages NONE , filed with the letter of

[X] the drawings:
pages 1 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of

[X] the sequence listing part of the description:
pages NONE , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained in the international application in printed form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages NONE

[X] the claims, Nos. NONE

[X] the drawings, sheets/~~fig~~ NONE

5. [ ] This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

*** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US99/31230

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step r industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	9, 11, 13-53	YES
	Claims	1-8, 10, 12	NO
Inventive Step (IS)	Claims	13-53	YES
	Claims	1-12	NO
Industrial Applicability (IA)	Claims	1-53	YES
	Claims	NONE	NO

2. citations and explanations (Rule 70.7)

Claims 1-8, 10 and 12 lack novelty under PCT Article 33(2) as being anticipated by Heather et al.

Heather et al. teach a broccoli cultivar 'XPH 5168' which is heat tolerant. It produces market acceptable broccoli heads at 35 degrees Celsius (95 degrees fahrenheit), (Table 4 and 5).

Claims 1-12 lack an inventive step under PCT Article 33(3) as being obvious over Heather et al. in view of Dufault.

Heather et al. teach heat tolerant broccoli which can tolerate a heat treatment of one week at 95 degrees fahrenheit (page 891, col. 1).

Heather et al. do not teach tolerance to a 15 day heat treatment of 85 degrees fahrenheit nor do they teach a method of tissue culture production for broccoli.

Dufault teaches that heat adversely affects floral development in broccoli and that heat tolerant broccoli would be useful for production of this crop during summer months in southeastern states (page 705, col. 1). Dufault teaches that 'Baccus' has acceptable color, bract number and compactness when grown at 32 degrees celsius (89.6 degrees fahrenheit) (page 708-709).

A skilled plant breeder would recognize the need to produce heat tolerant broccoli cultivars such as those taught by Heather et al. which can withstand even longer heat treatments because production of this crop in hot southeastern climates is desirable, as taught by Dufault. A plant breeder would want to reproduce such a heat tolerant plant by tissue culture to ensure that the trait is expressed in clonal offspring.

Claims 13-53 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest these specific broccoli cultivars.

Claims 1-53 meet the criteria set out in PCT Article 33(4), because heat tolerant broccoli cultivars would expand the regions

(Continued on Supplemental Sheet.)

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

CLASSIFICATION:

The International Patent Classification (IPC) and/or the National classification are as listed below:

IPC(7): A01H 5/00, 5/02, 5/04, 5/06, 5/08, 5/10, 5/12, 4/00; C12N 5/04 and US Cl.: 800/306, 298, 260, 278; 435/410, 430

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):

and seasons acceptable for production of this crop.

---------- NEW CITATIONS ----------

NONE

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(71) Applicant *(for all designated States except US)*: R & D AG INC. [US/US]; P.O. Box 2230, Gilroy, CA 95021 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: BARHAM, Robert [US/US]; 10030 New Avenue, Gilroy, CA 95020 (US). JOYNT, David [US/US]; 230 Barnes Lane, Hollister, CA 95023 (US).

(74) Agents: WARD, Michael, R. et al.; Limbach & Limbach L.L.P., 2001 Ferry Building, San Francisco, CA 94111–4262 (US).

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Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: HEAT TOLERANT BROCCOLI

(57) Abstract

Heat tolerant broccoli plants and seed produced therefrom are described. The heat tolerant broccoli plants are capable of producing a commercially acceptable broccoli head under heat stress growth conditions. The heat tolerant broccoli plants are exemplified by seeds deposited with the American Type Culture Collection and having ATCC Accession numbers: 203530, 203531, 203532, and 203533.

FOR THE PURPOSES OF INFORMATION ONLY

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HEAT TOLERANT BROCCOLI

FIELD OF THE INVENTION

This invention is in the field of plant breeding. In particular, this invention relates to the development of heat tolerant broccoli (*Brassica oleracea* L. var. *italica*).

BACKGROUND OF THE INVENTION

Broccoli (*Brassica oleracea* L. var. *italica*) has become an increasingly popular crop worldwide especially in health-conscious areas of the western world such as the North America, Europe, and Japan. An average broccoli stalk contains only 30 calories and provides 240% of the recommended daily allowance of vitamin C plus 10% of the recommended daily allowance of vitamin A. In addition to its nutritional value, some recent studies have shown that broccoli aids in the prevention of some forms of cancer.

Broccoli is a cool weather crop. High temperatures (>80°F) for even relatively short periods of time and warm temperatures (>75°F) for extended periods of time cause broccoli heads to be rough with uneven flower bud sizes and thus commercially unacceptable. {(Björkman, T., et al. (1998) High temperature arrest of inflorescence development in broccoli (*Brassica oleracea* var. *italica* L.) Journal of Experimental Botany 49:101-106.} As a result of the high sensitivity to heat during growth, broccoli can only be grown in limited areas under cool weather conditions.

Previous attempts at identifying heat tolerant broccoli cultivars have not been successful because broccoli is sensitive to relatively short periods of heat stress thereby making field observations too variable for effective genetic screening. Björkman, et al. (1998).

Thus, there is a need to develop heat tolerant broccoli varieties that will produce commercially acceptable broccoli heads under warm weather heat stress growth conditions. In addition, there is a need to develop heat tolerant broccoli inbred lines useful for producing heat tolerant F1 seed.

SUMMARY OF THE INVENTION

In order to meet these needs, the present invention is directed to heat tolerant broccoli plants. In particular, this invention is directed to broccoli seed capable of germinating and growing into a plant capable of producing a commercially acceptable head under heat stress growth conditions.

The broccoli seed of this invention are capable of germinating into a plant capable of producing a commercially acceptable head under heat stress growth conditions that render the heads of commercially available broccoli commercially unacceptable.

In addition to being heat tolerant, the broccoli seed of this invention are capable of germinating into a plant that is predominately mildew resistant.

The broccoli seed of this invention will produce a plant with a commercially acceptable head when the plant is exposed to a maximum temperature of 90°F for at least 5 consecutive days during the growth cycle; when the plants are exposed to a maximum temperature of at least 95°F for at least one day during the growth cycle; when the plants are exposed to a maximum temperature of 85°F for at least 15 days during the growth cycle; when the plants are exposed to a maximum temperature of at least 75° for at least 25 days during the growth cycle; when the plants are exposed to a maximum temperature of at least 80°C for at least 20 days during the growth cycle and other heat stress growth conditions.

The broccoli seed of this invention include but are not limited to those seeds designated M7028, M7007, M7009, M7022, 393-2-19, H7008, H7022, 393-2-47, 98-2192, 98-2088, 98-2061, H7007, H7028, H7010, and H7021R. The broccoli seed of this invention further include lines 4243, 4221, 4441, 4274-1, 4274-2, 4278-1, 4284-1, 4285-1, 4354-1, 4354-2, 4377-1, 4318-1, 4320-1, 4320-2, 4321-1, 4437-1, 4476-1, 4462-1, 4308-2, 4309-1, 4355-1, 4412-1, 4301, 4303, 4304, 4317, 4468, 4470, 4471, 4263-1, 4430-1, 4450-1, 4450-2, 4432-1, 4267-1, 7861, 7864, 7865, 7881, 7887, 7935, 8092, 7883, 7914, 7770, 7778.

The broccoli seed of this invention further include lines 4201, 4219, 4237, 4280, 4287, 4288, 4289, 4290, 4291, 4458-1, 4460-1, 4415, 4418, 4395-2.

Each of the lines of this invention can be crossed with other broccoli lines.

The broccoli seeds of this invention include inbred lines, hybrid lines, male lines and female lines, all of which are heat tolerant and capable of producing a commercially acceptable head under heat stress growth conditions.

This invention is further directed to broccoli plants or parts of broccoli plants produced from the broccoli seed of the invention. The invention is further directed to broccoli plants regenerated from tissue culture of the broccoli plants of this invention. The tissue culture of the invention comprises regenerable cells including meristematic tissue, anthers, leaves, ovules, roots, embryos, protoplasts and pollen and plants regenerated from these cells.

The invention is further directed toward transgenic heat tolerant broccoli plants. The transgenic heat tolerant broccoli lines may be resistant to various herbicides or pesticides.

The invention is further directed to broccoli plants having all of the phenotypic characteristics of the plants produced from the heat

tolerant broccoli seed of the invention. The invention is further directed to plants resulting from selecting, crossing, breeding or otherwise altering the broccoli plants of this invention.

The invention is further directed to biological material isolated from the plants of this invention. Such material includes but is not limited to RNA, DNA, protein and carbohydrate. The DNA of these plants includes the gene(s) involved in heat tolerance.

This invention is further directed to the seeds and plants produced from crossing other broccoli lines with plants grown from the seed of this invention.

This invention is further directed to methods of breeding heat tolerant broccoli lines.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will be better understood by reference to the drawings in which:

Figure 1 shows various broccoli head shapes. Broccoli heads are referred to as domed, semi-domed and deep-domed. The shapes of the various domes are (1) circular; (2) transverse broad elliptic; (3) transverse elliptic and (4) transverse elliptic narrow.

Figure 2 shows a cross section of eight 40-inch beds utilized for field production of the hybrid seed of this invention. In this diagram: F = female line seed-line; M1 = first male planting seed-line; M2 = second male planting seed-line; M3 = third male planting seed-line and M4 = fourth male planting seed-line.

DETAILED DESCRIPTION OF THE INVENTION

In order to more completely understand the invention, the following definitions are provided.

Broccoli: Broccoli (*Brassica oleracea* L. var. *italica*) is a cool season vegetable in the mustard family. Principal broccoli varieties currently grown in California include, in the coastal valleys, Everest, Greenbelt, Legacy, Marathon, Ninja, Olympia, Pinnacle, Pirate, Republic, Shogun, and Sultan; in the desert valleys, Arcadia, Captain, Emperor, Everest, Galaxy, Galleon, Greenbelt, Major, Marathon, Ninja, Packman, Patriot, Pirate, and Sultan; and in the San Joaquin Valley, Arcadia, Captain, Everest, Greenbelt, Legacy, Legend, Marathon, Pirate, and Republic. Varieties grown in the Pacific Northwest are: Arcadia, Emerald City, Excelsior, Pakman Patriot, Pirate, Regal, Arcadia, Buccaneer, Emerald City, Emperor, Everest, Excelsior, Green Belt, Green Valiant, Laguna, Legend, Liberty, Major, Marathon, Pakman, Patriot, Pinnacle, Pirate, Premium Crop, Regal, Shogun, Samurai, Triathlon, Windsor, Barbados, Embassy, Green Comet, Green Defender, HMX 1134, Idol. Because of heat sensitivity, broccoli is typically grown for harvest in the spring and fall.

Commercially Acceptable Broccoli: Commercially acceptable broccoli is broccoli which vegetable growers/shippers find acceptable for sale and consumers find acceptable for personal purchase and, ultimately, human consumption. Commercially acceptable broccoli has small uniform beads, good blue-green to green color, and tight, dome-shaped heads that extend above the leaves for ease of harvest. In commercial plantings under optimum conditions, large leafy broccoli plants produce a compact flower head on a tall, green, branching stalk. The center flower head is from 3 to 8 inches (7.5-20 cm) in diameter and plants average 24 to 36 inches (60-90 cm) tall. Hollow stems, water head rot, brown or yellow beads, bracts (leaflets) within heads, uneven bead size, and excessive branching

are undesirable and commercially unacceptable defects in broccoli that can be caused by exposure to heat.

Heat Tolerant Broccoli: Heat tolerant broccoli is broccoli that will produce a commercially acceptable product when grown under heat stress growth conditions for broccoli.

Heat Stress Growth Conditions: Heat stress growth conditions for broccoli are elevated temperature growth conditions that result in broccoli that exhibit heat stress symptoms that result in a commercially unacceptable product. Heat stress symptoms include non-uniform beads; brown, yellow, light-green or purple colored heads; loose flat heads; prominent leaflets that come through the broccoli head as bracts; hollow stems; water head rot and excessive branching.

Single Plant Selection: Single plant selection is the process of selecting single plants, which exhibit desired traits or characteristics. Seeds from the single plant are collected, stored and then grown in a subsequent growing period for further selections.

Massed: Broccoli plants are massed when a number of plants are selected and brought together for cross-pollination as a group. Massing prevents further inbreeding and tends to "fix" the broccoli line at the stage from which the selections were made.

Self-Pollination/Self-Pollinator: Self-pollination is the process of putting pollen from a plant onto a receptive female flower-part of that same plant. A plant that is a self-pollinator is a plant that accepts its own pollen to make seed that typically will give rise to

plants very similar or the same as the self pollinator plant. A plant that is self-pollinated is said to be selfed.

Self-Incompatible: A self-incompatible plant will not, under normal conditions, accept its own pollen nor generate any self-seed. Self-incompatible lines are generally designated "female." Self-incompatible lines are generally crossed with other lines to produce hybrid seed.

Self-Compatible: A self-compatible plant accepts its own pollen and will produce self-seed. Self-compatible lines are generally designated "male."

Progeny: Progeny is a broccoli line that is the offspring of the previous generation broccoli line.

Sessile: Attached to the stem by the base of the leaf.

Petiolate: Attached to the stem via a petiole.

Hybrid: The progeny of cross-fertilization between parents belonging to different genotypes.

Hybrid Vigor: The phenomenon in which the cross of two parent stocks produces hybrids that show increased vigor/heterosis compared to the parent stocks.

Inbred Lines: A nearly homozygous line produced by continuous inbreeding.

Pedigree Br eding: A system of breeding in which individual plants are selected in the segregating generations from a cross on the basis of their desirability and on the basis of a pedigree record.

The terminology used to describe the broccoli plants of this invention are generally those used by the Plant Variety Protection Office in PVP form STD-470-44 "Objective Description of Variety Broccoli (*Brassica oleracea* var. *italica*)." The following terminology is used herein in comparative study #1 and comparative study #2.

1. **REGION OF ADAPTATION (Area where best adapted in USA)**:

(1) Northwest; (2) NorthCentral; (3) Northeast; (4) Southeast; (5) Southwest; (6) Most regions and (7) Pacific Coast.

2. **MATURITY (Main Crop at 50% Harvest)**:

Harvest Season: (1) Fall; (2) Fall/Winter; (3) Winter/Spring; (4) Spring/Summer; (5) Summer; and (6) Summer/Fall.

Spring Planted: (1) Days from Direct Seeding to 50% Harvest; (2) Days from Transplanting to 50% Harvest; and (3) Length of Harvest Period in days.

Fall Planted: (1) Days from Direct Seeding to 50% Harvest; (2) Days from Transplanting to 50% Harvest; and (3) Length of Harvest Period in days.

Time of beginning of flowering (50% of plants with at least 10% flowers: (1) Early; (2) Med-Early; (3) Medium; (4) Med-Late; and (5) Late.

3. **SEEDLING**:

Cotyledon Color: (1) Yellow-Green; (2) Light Green; (3) Medium Green; (4) Dark Green; (5) Blue-Green; and (6) Purple-Green.

Cotyledon Anthocyanin: (1) Absent; (2) Weak; (3) Intermediate; and (4) Strong.

Hypocotyl Anthocyanin: (1) Absent; (2) Weak; (3) Intermediate; and (4) Strong.

4. **PLANT (At Harvest)**:

Plant Height: (cm) from soil line to top of leaves

Head Height: (cm) from soil line to top of head

Plant Branches: (1) Few; (2) Medium; and (3) Many.

Plant Habit: (1) Spreading; (2) Intermediate; and (3) Compact.

Market Class: (1) Fresh Market; (2) Processing; and (3) Both

Life Cycle: (1) Annual; (2) Biennial; and (3) Perennial.

Type of Variety: (1) Inbred; (2) Open-Pollinated; and (3) First generation Hybrid.

5. **OUTER LEAVES (at Harvest)**:

Number of Leaves per Plant:

Width at midpoint of plant including petiole:

Length at midpoint of plant including petiole:

Petiole Length:

Leaf Ratio-Length/Width: (1) (2:1); (2) (3:1); (3) (4:1); (4) (5:1); and (5) (6:1).

Leaf Attachment: (1) Sessile; (2) Petiolate; and (3) Sessile and Petiolate (both).

Wax Presence: (1) None; (2) Weak; (3) Intermediate; and (4) Strong.

Foliage Color (with wax if present): 1 Light Green; (2) Medium Green; (3) Dark Green; (4) Grey-Green; (5) Blue-Green; and (6) Purple-Green.

Leaf Shape: (1) Narrow Elliptic; (2) Elliptic; and (3) Broad Elliptic.

Leaf Base: (1) Blunt and (2) Pointed.

Leaf Apex: (1) Blunt and (2) Pointed.

Leaf Margins: (1) Straight; (2) Slightly Wavy; and (3) Very Wavy.

Leaf Veins: (1) Thin; (2) Intermediate; and (3) Thick.

Midrib: (1) Not Raised; (2) Slightly Raised; and (3) Raised.

Blistering (1) None; (2) Weak; and (3) Intermediate; and (4) Strong.

Attitude (Leaf Angle from Ground): (1) Horizontal (0-15 degrees); (3) Semi-erect (35-55 degrees); and (5) Erect (80-100 degrees).

Torsion of Leaf Tip: (1) None; (2) Weak; (3) Intermediate; and (4) Strong.

Profile of Upper Side of Leaf: (1) Concave; (2) Planar; and (3) Convex.

6. HEAD (At Market Maturity):

Diameter at widest point:

Depth:

Weight: market trimmed

Color: (1) Light Green; (2) Medium Green; (3) Dark Green; (4) Blue/Green; and (5) Purple.

Head Shape: (1) Circular; (2) Transverse Broad Elliptic; (3) Transverse Elliptic; and (4) Transverse Elliptic Narrow.

Dome Shape: (1) Domed; (2) Semi-domed; and (3) Deep Domed.

Head Size: (1) Small; (2) Medium; and (3) Large.

Compactness: (1) Long Pedicels (Loose); (2) Medium; and (3) Short Pedicels (Tight).

Surface Knobbling: (1) Fine; (2) Medium; and (3) Coarse.

Beads size: (1) Small; (2) Medium; and (3) Large.

Flower Buds: (1) Even in size; and (2) Uneven in size (cateye).

Anthocyanin Coloration: (1) Absent; 2 Present; (3) Leaf Axils; (4) Leaf Veins; (5) Leaf Blade; (6) Entire Plant; and (7) Leaf Petiole.

Color of Head Leaves: (1) White; (2) Green; (3) Red; and (4) Purple.

Secondary Heads: (1) Completely absent; (2) Basal; (3) Combination; and (4) Axillary along entire main stem up to main head.

Prominence of Secondary Heads: (1) Weak, (2) Intermediate; and (3) = Strong.

Number of Secondary Heads:

7. **COLOR**:

Flower Color: (1) White; (2) Cream; and (3) Yellow.

Flower Stalk Color: (1) Green; (2) Purple; and (3) Variegated.

8. **DISEASE RESISTANCES**:
1 = Most Susceptible
5 = Intermediate
9 = Most Resistant

Black Leg (*Leptosphaeria maculans*)	Black Leg
Black Spot (*Alternaria* spp.)	Black Spot
Black Rot	Black Rot
Bottom Rot (*Rhizoctonia solani*)	Bottom Rot
Cauliflower Mosaic Virus	Cauliflower Mosaic Virus
Cerospora Leaf Spot (*Cercospora brassicicola*)	Cerospora Leaf Spot
Clubroot (*Plasmodiophora brassicae*)	Clubroot
Downy Mildew (*Peronospora parasitica*)	Downy Mildew
Erwinia Sp.	Erwinia Sp.
Phytophthora Root Rot (*Phytophthora megasperma*)	Phytophthora Root Rot
Powdery Mildew (*Erysiphe polygoni*)	Powdery Mildew
Pseudomonas	Pseudomonas
Ring Spot (*Mycosphaerella brassicicola*)	Ring Spot

Turnip Yellow Mosaic Virus	Turnip Yellow Mosaic Virus
Verticillium wilt (*Verticillium albo-atrum*)	Verticillium wilt
White Blister (*Albugo candida*)	White Blister
Xanthomonas campetis	Xanthomonas campetis
Yellows (*Fusarium oxysporum*)	Yellows

9. **OTHER RESISTANCE**:

1 = Most susceptible
5 = Intermediate
9 = Most Resistant

Insect
Buttoning
Blindness
Bolting
Brown beads
Drought
Cold
Hollow Stem
Riceyness
Whiptail

10. **HEAT TOLERANCE**:

Heat tolerance was measured on a scale of 1-9 with 9 being the most heat tolerant and 1 being the least heat tolerant. For heat tolerance, ratings of five (5) or below are indicative of a broccoli plant that produced a commercially unacceptable head. A rating of six (6) is indicative of broccoli plants that exhibit no heat stress symptoms when exposed to 90°F. A rating of seven (7) is indicative of broccoli plants that exhibit no heat stress symptoms when exposed to 95°F. A rating of eight (8) is indicative of broccoli plants that exhibit no heat stress symptoms when exposed to 100°F. A rating of nine (9) is indicative of broccoli plants that exhibit no heat stress symptoms when exposed to 105°F. In some studies, plants were not exposed to a temperature of 105°F so that a rating of 9.0 could not be assigned.

Under some circumstances, the heat tolerant ratings are followed by a (+) or (-) to indicate a plant exhibiting symptoms

slightly better (+) or slightly worse (-) than the assigned number. In other circumstances, the ratings are presented as a fraction of a rating number. For example, a rating of 7.1 is slightly better than a rating of 7.0. A rating of 7.5 is half way between a rating of 7.0 and 8.0. A rating of 6.8 is slightly worse than a rating of 7.0. A slightly higher rating means that the heat stress symptoms were slightly less evident. Similarly, a slightly lower rating means that the heat stress symptoms were less evident.

Taking into account these definitions, the present invention is directed to heat tolerant broccoli plants. The heat tolerant broccoli of this invention is capable of producing a commercially acceptable product when grown under heat stress conditions.

Heat stress is exhibited in broccoli by a number of different symptoms. These symptoms include non-uniform beads; brown, yellow, light-green or purple colored heads; flat heads; bracts (leaflets in the head); rapid fracturing of the head, which reduces the harvest period; "cateye" (death of growing points), extremely small heads, and hollow stems.

Each of these symptoms is generally viewed as commercially unacceptable. The greater the number of heat stress symptoms, the more commercially unacceptable the broccoli plant. Heat stress symptoms in broccoli result from a number of interacting factors. The most important of these interacting factors are the temperature, the duration of the high temperature exposure (hours, days, weeks), the available soil moisture supply and the wind speed. Of critical importance is the timing of the exposure to the heat stress conditions during the growth cycle of broccoli. It has been shown that heat stress of broccoli can be due to an inhibition of the enlargement of broccoli bud primordia. Broccoli buds are not as sensitive to heat once they differentiate. The different heat sensitivity and resulting contrast between the delayed buds and the

unaffected buds causes the uneven head appearance under heat stress growth conditions. If heat stress occurs prior to bud development (i.e., during vegetative development) no injury is generally seen. If the heat stress is applied late in bud development, many buds are affected but these buds can be obscured by the older buds.

Because of extreme sensitivity to heat stress, broccoli grown in the spring and the fall, when cooler temperatures are the norm, are at less risk of heat stress. However, a single day during the spring or fall with a high temperature of 100°F or several (2-3) warmer days (>80°F) or multiple (5-7) warm days (>75°F) at the critical point during broccoli bud development can render an entire field so damaged by heat stress that none of the heads are commercially acceptable.

The present invention is thus directed toward the development of heat tolerant broccoli varieties and hybrids. The broccoli varieties and hybrids of this invention will produce commercially acceptable heads when the plants are grown during heat stress growth conditions during late spring, summer, and early fall in California, Arizona, Mexico, and many other areas traditionally considered to be too warm for broccoli growth, or at risk of heat stress.

Broccoli Production

Broccoli may be grown by transplant production or by direct seeding. For transplant production, plants may be started in hotbeds or greenhouses. Broccoli seedlings grown in a hotbed need a loose, easily pulverized loam that is not too fertile. If the plants are started in hotbeds, soil fumigation is needed to control weeds, soil borne diseases, and insects. Seeds are planted one-quarter to one-half inch deep in rows 4 to 6 inches apart with 2 to 4 seeds per inch and covered with a sash or plastic covering. The seedlings are thinned

at the two-leaf stage allowing 1½ inches between plants. Plants are watered twice daily and fertilized with soluble fertilizer at least every 2 weeks. Proper ventilation is important and can be maintained by raising the sash or plastic covering during the hottest portion of the day. In the hotbed, if properly handled, 3 or 4 ounces of seed will produce enough seedlings to plant 1 acre. When seed is planted in beds, it generally requires about 6 to 8 weeks from seed to plants for the spring crop, and about 4 to 5 weeks for the fall crop.

In the greenhouse, a variety of plant growing containers may be used (i.e. plastic cell packs, peat pots, and speedling trays) for growing broccoli. These containers can be filled with an artificial media, usually a combination of peat, perlite, vermiculite, and in some instances bark. The seeds can be sown directly into the containers and thinned upon emergence to 1 plant per cell or pot. In the greenhouse, it generally requires 5 to 6 weeks from seed to plants for the spring crop and 4 to 5 weeks for the fall crop.

For direct seeding, broccoli seeds may be direct seeded in the field using a precision planter. Seed required for one acre is generally 0.75 to 1.25 pound when using a precision seeder.

ORIGIN AND PARENTAGE OF HEAT TOLERANT BROCCOLI PLANTS

The broccoli of this invention were created by classical plant breeding as well as anther culture techniques. The breeding history of the "inbred," "self compatible" and "male" lines identified are exemplified by the following breeding histories.

A. DEVELOPMENT OF INBRED LINES

Numerous heat tolerant inbred lines were developed. For illustrative, but nonlimiting purposes, the breeding histories of the following inbred lines are presented. Unless otherwise noted, single

plant selections were made each year for plants exhibiting heat tolerance.

1. Inbred Lines 393-2-19, 393-2-47, and 393-2-32

All lines designated "393-2-XX" where XX represents a different number for a line were isolated and developed as indicated below. Representative lines include 393-2-19, 393-2-47, 393-2-32.

Fall, Year 1	Commercial broccoli hybrid Marathon was crossed with broccoli hybrid "No. 608" obtained from IM Foods, Incorporated, Gilroy, California.
Summer, Year 2	F1 seed from Marathon x No. 608 were planted into row number 393 of a summer broccoli trial in Gilroy, California, and single plant selections were made for heat tolerance and downy mildew resistance.
Fall, Year 2	Single plant selection number 2 from row 393, i.e. "393-2", which had exhibited good heat tolerance and downy mildew resistance, was entered into anther culture.
Spring, Year 3	Anther culture product numbers 19 and 47 from 393-2, i.e."393-2-19," "393-2-47" and "393-2-32" were transplanted into the greenhouse in Gilroy, California. 393-2-19, 393-2-47 and 393-2-32 were observed to exhibit desirable horticultural traits for deep dome-shaped head, lack of side shoots, good vigor, and high yield. 393-2-19, 393-2-47 and

393-2-32 also demonstrated ability to produce self-pollinated seed.

Summer, Year 4	The original seed from 393-2-19, 393-2-47 and 393-2-32 made in the greenhouse in Spring, year 3, were seeded in the greenhouse in Summer, year 4, and subsequently transplanted to the field for evaluation in Gilroy, California, in the summer. 393-2-19 and 393-2-47 exhibited outstanding uniformity and were considered breeding true as a spontaneously doubled-haploid, "inbred", line. Plants were taken from the field plot for self-pollination and subsequent seed increase.
From Year 5 to Present	393-2-19, 393-2-47 and 393-2-32 have consistently exhibited exceptionally good uniformity and stability through generations of seed increase with no variants or off types plants ever observed.

In the breeding history described above, commercial broccoli hybrid Marathon was crossed with broccoli hybrid No. 608 obtained from IM Foods, Incorporated, Gilroy, California. The commercially available broccoli hybrid Marathon was selected for the initial cross because it had demonstrated good yield potential. Hybrid No. 608 was selected for the initial cross because it was thought to have less side shoots, an advantageous characteristic for harvesting.

During the breeding process, F1 seed from Marathon x No. 608 were planted and grown. Selections were made for heat tolerance and downy mildew resistance. The heat tolerance

selection was conducted at head formation through harvest maturity. The selection criteria were smooth domed head, even flower-bud size, good head color, lack of bracting (leaflets in the head), and an ability to hold a good head shape through harvest maturity. The downy mildew selection was conducted throughout the growth cycle of the plants. The selection for downy mildew resistance was based on plants with no mildew lesions or a greatly reduced number of lesions present on any leaves as compared to non-resistant plants.

Multiple single plant selections exhibiting heat tolerance and downy mildew resistance were entered into anther culture. Anther culture procedures are well known in the art of plant breeding. In anther culture techniques, the undifferentiated pollen mother cells that exist in immature broccoli anthers are stimulated *in vitro* into embryonic states by procedures well known in the literature. The undifferentiated pollen mother cells may be subjected to treatments of higher temperatures, light and dark and specialized media growth conditions including hormone simulation. Plant growth conditions of 60°F and bright light followed by a heat shock of 90°F after anther excision and culturing can help stimulate embryogenisis. This process can stimulate the development of embryonic growth wherein the haploid (one-half the chromosome number) pollen mother cell multiplies and grows into callus tissue. The callus tissue, through the use of specialized media, hormone treatments, and controlled temperature and light can be stimulated to make green plant shoots and eventually functional roots. Some of these haploids spontaneously double their chromosome number, thus; generating "di-haploids," which are essentially completely homozygous. These highly homozygous lines are genetically equivalent to the end result of many years of self-pollination by conventional means.

In a preferred format, anther cultures are prepared as described in Keller, et al., Embryogenesis and Plant Regeneration in

Brassica napus Anther Cultures, *Canadian Journal of Botany* 55: 1383-1388 (1977); Keller, et al. Production of Haploids via Anther Culture in Brassica Oleracea Var. Italica, *Euphytica* 32: 151-159 (1983); and Orton, et al., Segregation of Genetic Markers among Plants Regenerated From Cultured Anthers of Broccoli (Brassica oleracea var. 'italica') *Theor Appl Genet* 69:637-643 (1985).

In one format, broccoli anthers are prepared and cultured as follows. Immature broccoli racemes are removed from broccoli heads as they begin to elongate, but before the first buds are opened. Racemes are then sterilized, for example in 20% W:V household bleach for 15 minutes under agitation with one drop of detergent per 100ml as a surfactant. The racemes are then washed at least three times with sterile distilled water for generally 10 minutes per wash. Anthers are then generally removed by carefully peeling back the immature calyx and corolla and gently rupturing the point of filament attached to the anther axis. Care must be taken to minimize damage to the anther culture. Anthers are then placed into liquid culture medium as described in Keller, et al. (1977). Such culture medium may include L-serine at a concentration of 100mg/l. Anthers are cultured at, for example, 35°C for 36h and transferred to 25° (all in the dark). In some circumstances, anther-derived embryos are then kept in continuous fluorescent light (25°C) for 1 week to permit greening and then transferred to hormone-free solidified B_5 medium as described by Gamborg, et al. *Exp. Cell Res.* 50: 151-158 (1968). Upon transfer to the hormone-free medium, the anther-derived embryos are cultured at 25°C in light. Embryos which fail to develop into plantlets may be cut into sections and cultured on a modified Murashige and Skoog medium [Murashige, et al., *Physiol. Plant* 15: 473-497 (1962)] containing 0.8% agar, 2% sucrose, 5 X 10^{-6} M benzyladenine and 10^{-7} M napthaleneacetic acid (NAA) to induce shoot regeneration. To induce rooting, developing

shoots are excised and cultured on B_5 medium in, for example, 60ml sterilin bottles. Rooted plantlets may then be planted in Jiffy-7 peat pellets and kept moist in a mist chamber. After 2 weeks, the broccoli plants can then be transferred to soil and grown in the greenhouse for further selections.

Once transferred to the greenhouse, various plants were selected based on desired phenotypical characteristics including an ability to produce self-pollinated seed. Self-pollination is advantageous because it permits seed increase and bulking of seed without random cross-pollination.

2. **Inbred lines: 4243, 4221 and 4441**

Using the procedures generally outlined above for 393-2-19 and 393-2-47, inbred lines 4243, 4221 and 4441 were isolated following the pedigree chart outlined below. IM Foods 608, Cruiser, Marathon and Sprinter are commercially available lines. Unless otherwise noted, all selections were single plant selections for heat tolerance. 393-2-19 is the same inbred line described above. Each season, the single plant selection exhibiting heat tolerance were selfed and seed was harvested for the next growing season.

Derivation of: 4243; 4221; and 4441

Year 1	IM Foods 608 X Cruiser	and	Marathon X IMF 608
	↓		↓
Year 2	Backcross F_1 to Cruiser		Self
	↓		↓
Year 3	Backcross BC_1 F_1 and Cruiser		F_2 Selection goes to Anther Culture
	↓		↓

	Year 4	Line 5334-1	X	Sprinter F_1 393-2-19 (a di-haploid anther culture product)
		↓		
	Year 5	Line 5526-1	X	393-2-19
			↓	
	Year 6		Self	
			↓	
	Year 7		Self	
			↓	
	Year 8		Self	
			↓	
	Year 9		Self	
			↓	
	Year 10		Self	

3. **Inbred lines: 4274-1; 4274-2; 4278-1; 4284-1; 4285-1; 4354-1; 4354-2; 4377-1**

Inbred lines 4274-1; 4274-2; 4278-1; 4284-1; 4285-1; 4354-1; 4354-2; 4377-1 were isolated following the pedigree chart outlined below using the procedures generally described above for inbred lines 393-2-19 and 393-2-47. Unless otherwise noted, single plant selections were made for heat tolerance.

Derivation of: 4274-1; 4274-2; 4278-1; 4284-1; 4285-1; 4354-1; 4354-2; 4377-1

Year 1	IMF608	X	Marathon
			↓
Year 2			Self
			↓
Year 3			Anther Culture
			↓
Year 4			Anther Culture Selection 393-2-47
			↓
Year 5			Unknown Outcross
			↓
Year 6			Self
			↓
Year 7			Self
			↓
Year 8			Self
			↓
Year 9			Self
			↓
Year 10			Self

4. Inbred lin s: 4318-1; 4320-1 and 4320-2; 4321-1; 4437-1; 4476-1; 4462-1

Inbred lines 4318-1; 4320-1 and 4320-2; 4321-1; 4437-1; 4476-1; 4462-1 were isolated following the pedigree chart outlined below using the procedures generally outlined above for inbred lines 393-2-19 and 393-2-47. Commercially available line Marathon was selfed. Repeated single plant selections for heat tolerance were made to produce the resulting lines. In year 6, the selected line was mass pollinated.

Derivation of: 4318-1; 4320-1 and 4320-2; 4321-1; 4437-1; 4476-1; 4462-1

Year 1	Marathon	Self
		↓
Year 2		Self
		↓
Year 3		Self
		↓
Year 4		Self
		↓
Year 5		Self
		↓
Year 6		Mass Pollinate
		↓
Year 7		Self

5. **Inbred lines: 4308-2; 4309-1; 4355-1; 4412-1; 4301; 4303; 4304; 4317; 4468; 4470; 4471**

Inbred lines 4308-2; 4309-1; 4355-1; 4412-1; 4301; 4303; 4304; 4317; 4468; 4470; 4471 were isolated following the pedigree chart outlined below using the techniques generally outlined above for inbred lines 393-2-19 and 393-2-47. In year 9, the selected lines were brush pollinated, i.e., pollinated with a brush. Single plant selections were made for heat tolerance.

Derivation of: 4308-2; 4309-1; 4355-1; 4412-1; 4301; 4303; 4304; 4317; 4468; 4470; 4471

Year 1: Cruiser X Green Belt

↓

Year 2: CGB X Marathon

↓

Year 3: Self

↙ ↘

Year 4: F_2 Selection 1 X F_2 Selection 2

↓

Year 5: Self

↓

Year 6 Self

↓

Year 7 Self

↓

Year 8 Self

↓

Year 9 Brush Group (open pollinated)

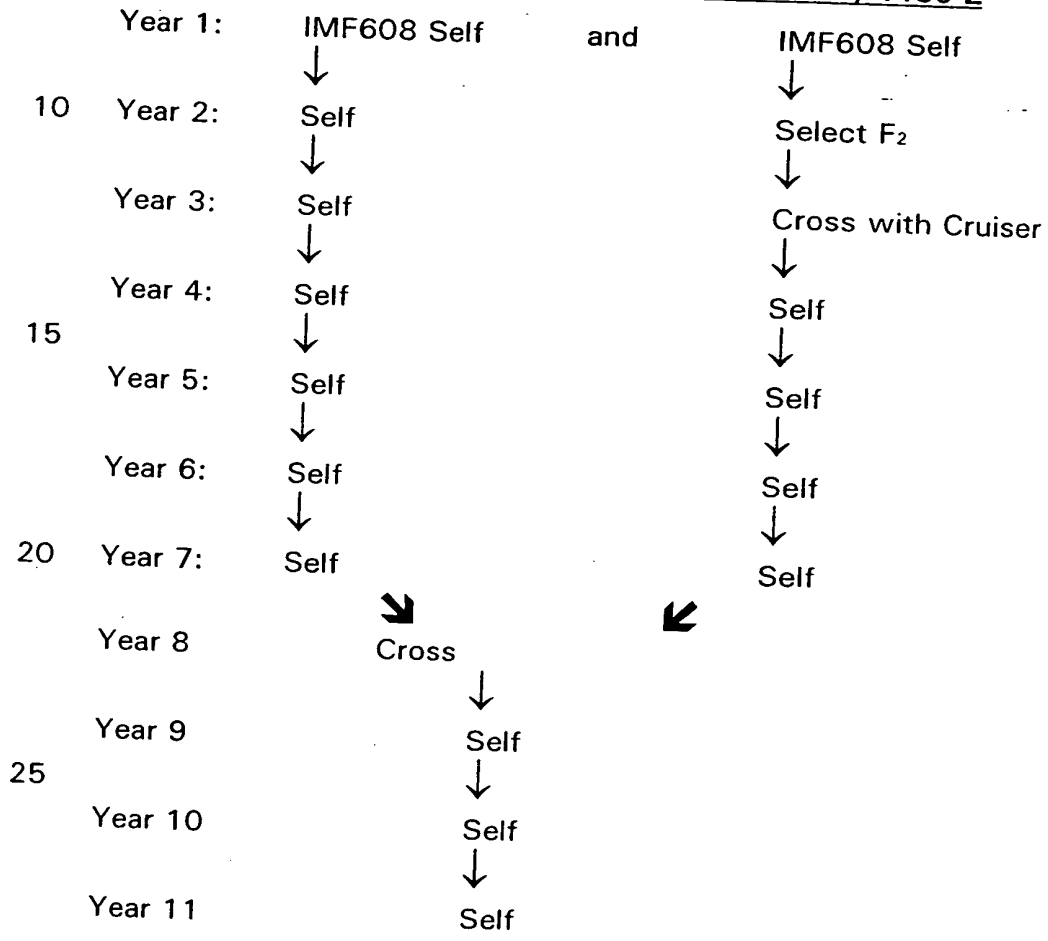
↓

Year 10 Self

6. **Inbred lines 4263-1; 4430-1; 4450-1 and 4450-2:**

Inbred lines 4263-1; 4430-1; 4450-1 and 4450-2 were isolated following the pedigree chart outlined below using the techniques generally outlined above for inbred lines 393-2-19 and 393-2-47. Single plant selections were made for heat tolerance.

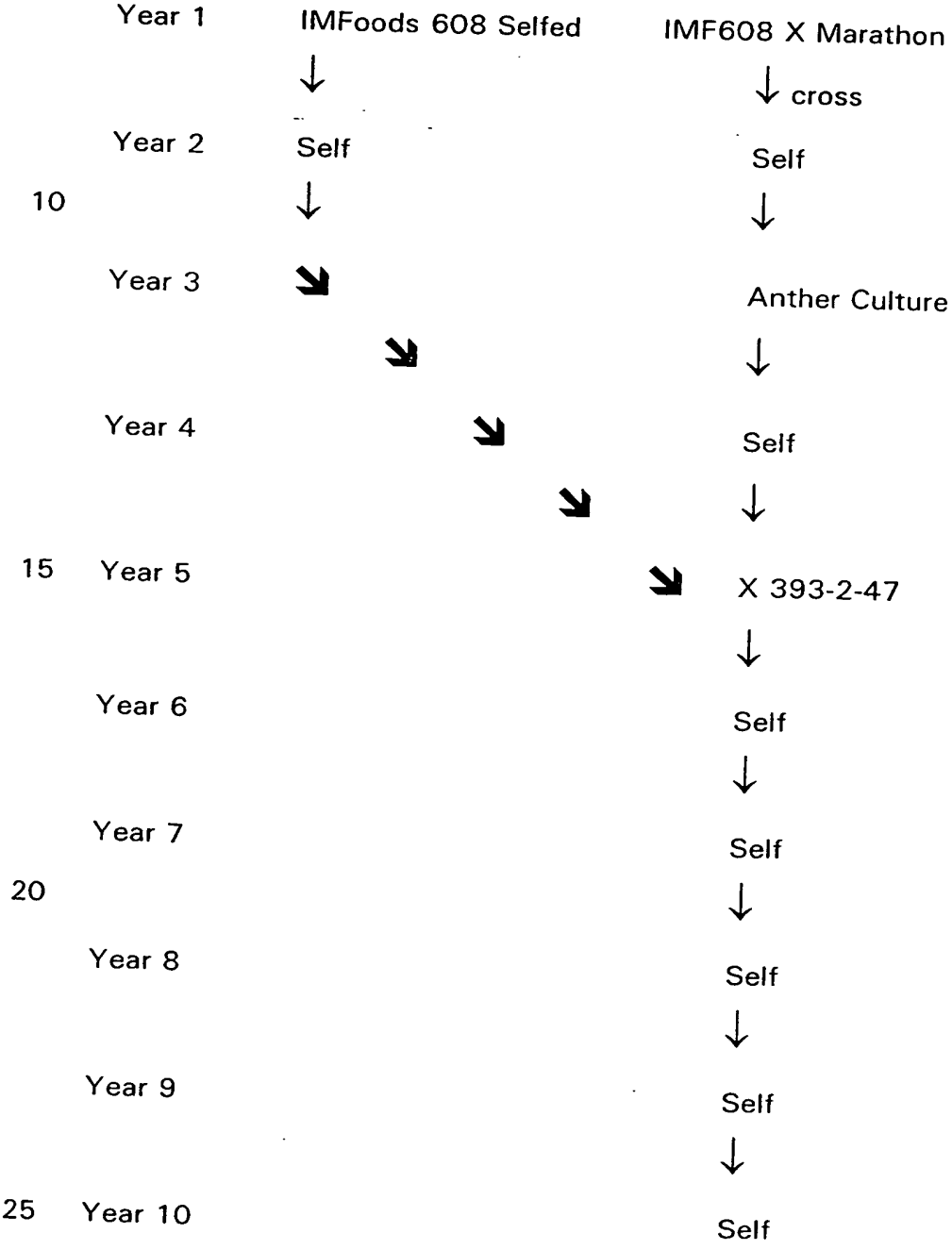
Derivation of: 4263-1; 4430-1; 4450-1; 4450-2



7. **Inbred line 4432-1**

Inbred line 4432-1 was isolated following the pedigree chart outlined below using the techniques generally outlined above for inbred lines 393-2-19 and 393-2-47. Single plant selections for heat tolerance were made.

Derivation of: 4432-1



8. **Inbred line 4267-1 (= "2192")**

Inbred line 4267-1 (= "2192") was isolated following the pedigree chart outlined below using the techniques generally outlined above for inbred line 393-2-19. Single plant selections were made for heat tolerance.

Derivation of: 4267-1 (= "2192")

	Year 1	Cruiser X IMF608
		↓
	Year 2	Self
		↓
	Year 3	Self
		↓
	Year 4	Self
		↓
	Year 5	Self
		↓
	Year 6	Self
		↓
	Year 7	Open-pollinate

9. **Inbred lin 7861**

Inbred line 7861 was isolated following the pedigree chart outlined below using the techniques generally outlined for inbred lines 393-2-19 and 393-2-47 outlined above. Single plant selections were made for heat tolerance.

Derivation of: 7861

Year 1	IMF 608	X	Southern Comet
		↓	
Year 2		X	Green Belt
		↓	
Year 3		X	Green Belt
		↓	
Year 4		Self	
		↓	
Year 5		Self	
		↓	
Year 6		Self	
		↓	
Year 7		Self	
		↓	
Year 8		Self	

10. Inbred line 7864

Inbred line 7864 was isolated following the pedigree chart outlined below using the techniques generally outlined for inbred lines 393-2-19 and 393-2-47 outlined above. Single plant selections were made for heat tolerance.

Derivation of: 7864

Year 1	Cruiser	X	Green Belt
		↓	
Year 2		X	Marathon
		↓	
Year 3		Self	
		↓	
Year 4		Self	
		↓	
Year 5		Self	
		↓	
Year 6		Self	
		↓	
Year 7		Self	
		↓	
Year 8		Self	

11. **Inbred line 7865**

Inbred line 7865 was isolated following the pedigree chart outlined below using the techniques generally outlined for inbred lines 393-2-19 and 393-2-47 outlined above. Single plant selections were made for heat tolerance.

Derivation of: 7865

Year 1	Synergene 6237	Self
	↓	
Year 2	Self	
	↓	
Year 3	Self	
	↓	
Year 4	Self	
	↓	
Year 5	Self	
	↓	
Year 6	Self	
	↓	
Year 7	Self	

12. Inbr d line 7881 and 1551

Inbred lines 7881 and 1551 were isolated following the pedigree chart outlined below using the techniques generally outlined for inbred lines 393-2-19 and 393-2-47 outlined above. Single plant selections were made for heat tolerance.

Derivation of: 7881 and 1551

Year 1	IMF 608 X	Green Belt
	↓	
Year 2	X	Green Belt
	↓	
Year 3	Self	
	↓	
Year 4	Self	
	↓	
Year 5	Self	
	↓	
Year 6	Self	
	↓	
Year 7	Self	
	↓	
Year 8	Self	
	↓	
Year 9	Self	

13. Inbred lin 7887

Inbred line 7887 was isolated following the pedigree chart outlined below using the techniques generally outlined for inbred lines 393-2-19 and 393-2-47 outlined above. Single plant selections were made for heat tolerance.

Derivation of: 7887

Year 1	IMF 608	Self	
		↓	
Year 2		X	Cruiser
		↓	
Year 3		Self	
		↓	
Year 4		Self	
		↓	
Year 5		Self	
		↓	
Year 6		Self	
		↓	
Year 7		Self	
		↓	
Year 8		Self	
		↓	
Year 9		Self	

14. **Inbred line 7935**

Inbred line 7935 was isolated following the pedigree chart outlined below using the techniques generally outlined for inbred lines 393-2-19 and 393-2-47 outlined above. Single plant selections were made for heat tolerance.

Derivation of: 7935

Year 1	Seoul Star	X	IMF 608
		↓	
Year 2		Self	
		↓	
Year 3		Self	
		↓	
Year 4		Self	
		↓	
Year 5		Self	
		↓	
Year 6		Self	
		↓	
Year 7		Self	
		↓	
Year 8		Self	

15. Inbred lin 8092

Inbred line 8092 was isolated following the pedigree chart outlined below using the techniques generally outlined for inbred lines 393-2-19 and 393-2-47 outlined above. Single plant selections were made for heat tolerance.

Derivation of: 8092

Year 1	Cruiser	X	Green Belt
		↓	
Year 2		X	Marathon
		↓	
Year 3		Self	
		↓	
Year 4		Self	
		↓	
Year 5		Self	
		↓	
Year 6		Self	
		↓	
Year 7		Self	
		↓	
Year 8		Self	
		↓	
Year 9		Self	

16. **Inbred line 7883**

Inbred line 7883 was isolated following the pedigree chart outlined below using the techniques generally outlined for inbred lines 393-2-19 and 393-2-47 outlined above. Single plant selections were made for heat tolerance.

Derivation of: 7883

Year 1	IMF 608	Self
	↓	
Year 2	Self	
	↓	
Year 3	Self	
	↓	
Year 4	Self	
	↓	
Year 5	Self	

17. Inbred line 7914

Inbred line 7914 was isolated following the pedigree chart outlined below using the techniques generally outlined for inbred lines 393-2-19 and 393-2-47 outlined above. Single plant selections were made for heat tolerance.

Derivation of: 7914

Year 1	Cruiser	X	Green Belt
		↓	
Year 2		X	IMF 608
		↓	
Year 3		X	Green Belt
		↓	
Year 4		Self	
		↓	
Year 5		Self	
		↓	
Year 6		Self	
		↓	
Year 7		Self	
		↓	
Year 8		Self	
		↓	
Year 9		Self	

18. Inbred lines 7770 and 5580-2

Inbred lines 7770 and 5580-2were isolated following the pedigree chart outlined below using the techniques generally outlined for inbred lines 393-2-19 and 393-2-47 outlined above. Single plant selections were made for heat tolerance.

Derivation of: 7770 and 5580-2

Year 1 Cruiser X Green Belt

↓

Year 2 X Marathon; IMF 608 Self

↘ ↙

Year 3 X ; IMF 608 X Arcadia

↘ ↙

Year 4 X

↓

Year 5 Self

↓

Year 6 Self

↓

Year 7 Self

↓

Year 8 Self

↓

Year 9 Self

↓

Year 10 Self

19. **Inbred line 7778**

Inbred line 7778 was isolated following the pedigree chart outlined below using the techniques generally outlined for inbred lines 393-2-19 and 393-2-47 outlined above. Single plant selections were made for heat tolerance.

Derivation of: 7778

Year 1	IMF 608	Self
	↓	
Year 2	Self	
	↓	
Year 3	Self	
	↓	
Year 4	Self	
	↓	
Year 5	Self	
	↓	
Year 6	Self	
	↓	
Year 7	Self	
	↓	
Year 8	Self	

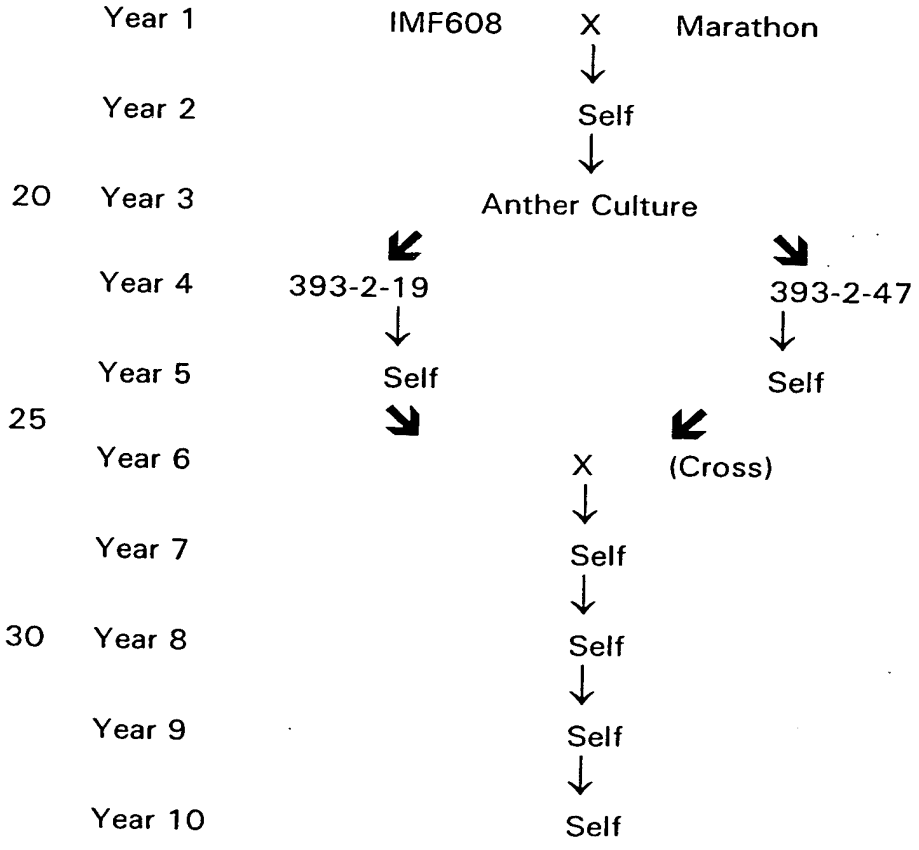
B. SELF INCOMPATIBLE LINES

Numerous heat tolerant self-incompatible ("female") lines were developed. For illustrative, but not limiting purposes, the breeding histories of the following self-incompatible lines are presented. Unless otherwise noted, single plant selections were made for heat tolerance.

1. Self-incompatible lines: 4201; 4219, 4237, 4280, 4287, 4288, 4289, 4290, 4291, 4458-1, 4460-1

Broccoli lines 4201; 4219, 4237, 4280, 4287, 4288, 4289, 4290, 4291, 4458-1, 4460-1 were isolated following the pedigree chart outlined below using the procedures generally outlined above for the isolation of 393-2-19.

Derivation of: 4201; 4219, 4237, 4280, 4287, 4288, 4289, 4290, 4291, 4458-1, 4460-1



2. **Self Incompatible Line: 4415**

Broccoli line 4415 was isolated following the pedigree chart outlined below using the procedures generally outlined above for 393-2-19.

Derivation of: 4415

Year 1	IMF608 X Green Belt
	↓
Year 2	X Green Belt
	↓
Year 3	Self
	↓
Year 4	Self
	↓
Year 5	Self
	↓
Year 6	Self
	↓
Year 7	Self
	↓
Year 8	Self
	↓
Year 9	Self
	↓
Year 10	Open-pollinate

3. **Self Incompatible Line: 4418**

Broccoli line 4418 was isolated following the pedigree chart outlined below using the procedures outlined above for 393-2-19.

Derivation of: 4418

Year 1	Green Belt X Cruiser		
	↓		
Year 2	X Marathon		Shogun X IMF608
	↘		↙
Year 3		Cross	
		↓	
Year 4		Self	
		↓	
Year 5		Self	
		↓	
Year 6		Self	
		↓	
Year 7		Self	
		↓	
Year 8		Self	

4. **Self Incompatible Line 4395-2**

Broccoli line 4395-2 was isolated following the pedigree chart outlined below using the procedures generally outlined above for 4935-2.

Derivation of: 4395-2

Year 1	Synergene 6236 Selfed
	↓
Year 2	Self
	↓
Year 3	Self
	↓
Year 4	Self
	↓
Year 5	Self
	↓
Year 6	Self
	↓
Year 7	Self
	↓
Year 8	Open pollinated
	↓
Year 9	Self

C. MALE LINES

Numerous heat tolerant "male" broccoli lines have been identified and shown stable and uniform. For illustrative but non-limiting purposes, the breeding histories of the M7007, M7009 and M7028 are provided as follows.

The "Cruiser" broccoli line was selected for initial crosses because it was a commercially available hybrid that showed a small degree of heat tolerance which was rated at approximately 5 and also had a nicely elevated head.

M7007

Year One	IM Hybrid No. 608 obtained from IM Foods, Incorporated, Gilroy, California, was self-pollinated.
Year Two	F2 of Hybrid No. 608 is crossed with Cruiser, which was obtained from Royal Sluis, a Dutch seed company.
Year Three	Heat tolerant single plant selection of the F2 Hybrid 608/Cruiser with heat tolerance equaling 8- was made.
Year Four	Heat tolerance equaling 7 + single plant selection gives [(No. 608) F2/Cruiser] F3.
Year Five	Eight selections are selfed and massed selected to give [(No. 608) F2/Cruiser] F4.
Year Six	Twelve selections are massed.
Year Seven	Fifteen selections are massed.
Year Eight	Five selections are massed selected and entered into a large isolation cage increase to give the finished line M7007.

M7009

Year One	IM Hybrid No. 608 is self-pollinated.
Year Two	F2 of Hybrid No. 608 is crossed with Cruiser.
Year Three	Heat tolerant single plant selection of the F2 Hybrid 608/Cruiser with heat tolerance equaling 8- was made.
Year Four	Heat tolerance equaling 7 + single plant selection gives [(No. 608) F2/Cruiser] F3.
Year Five	Eight selections are selfed and massed to give [(No. 608) F2/Cruiser] F4.
Year Six	Twelve selections are massed.
Year Seven	Fifteen selections are massed.
Year Eight	Five selections are massed selected and entered into a large isolation cage increase to give the finished line.
Year Nine	Seed storage.
Year Ten	Six selections are massed selected and entered into a large isolation cage increase to give finish line M7009.

M7028

Year Two	F2 of Hybrid No. 608 is crossed with Cruiser.
Year Three	Heat tolerant single plant selection of the F2 Hybrid 608/Cruiser with heat tolerance equaling 8- was made.
Year Four	Made single plant selection. [(No. 608) F2/Cruiser] F3
Year Five	Made single plant selection with heat tolerance equaling 7. [(No. 608) F2/Cruiser] F4
Year Six	Made single plant selection with heat tolerance equaling 7. [(No. 608) F2/Cruiser] F5
Year Seven	Selected five plants, massed selected and entered into a large isolation cage to give finished line M7028.

The male lines of this invention can be crossed with female lines (self-incompatible) to produce hybrid seed. The female lines

may or may not be heat tolerant. Encompassed within the scope of this invention are the hybrid seed produced from crossing the male lines of this invention with other broccoli lines of interest. Hybrid seed includes but is not limited to H7007, H7008, H7028.

HYBRID SEED PRODUCTION

For hybrid seed production of heat tolerant broccoli seed, two lines are selected for production. The lines are designated male or female, with the female being the recipient of the male line pollen. Either the male or female or both lines may be heat tolerant as defined by this invention. Broccoli plants flower with both the female and male parts and are capable of self-pollination. The line designated "female" is generally "self-incompatible," which means it will not accept its own pollen, a process developed in the plant by breeding. The line designated "male" is generally "self-compatible" and will accept its own pollen. Since self-incompatible lines will not accept their own pollen, but will out-cross with other broccoli pollen. Self-incompatible lines produce the commercially desired hybrid seed. The male line is the pollen provider to the female line. The cross of the self-compatible male line and the self-incompatible female line will produce a seed that is a hybrid.

Once a hybrid has been selected for seed production, a "nick" study is done. The nick study identifies the flowering period of the female, i.e. when it will start to flower and for how long it will flower. A nick study is also done for the male line and the two are compared. The nick study gives the data needed to determine if the female will require 1, 2, 3, or 4 male planting dates to cover its full flowering period.

Once the data from the nick study is obtained, seed of the female and the first male are planted in the greenhouse. The second male is planted in the greenhouse 7 – 10 days later, with the third

male planted another 7 – 10 days after that, and the final or 4th male planted within 10 more days. The female is seeded in the greenhouse at approximately 11,000 plants for each production acre and each male planting at 7,000 plants. Forty-five days from being planted in the greenhouse the female and first male are ready for transplanting in the field. The three remaining males are each transplanted into the field within 45 days of their individual greenhouse planting dates.

Field Production of Hybrid Seed

Field production of the hybrid seed is begun when all of the female plants and the first male plants from the greenhouse are transplanted into the field. Transplanting can be done by machine or by hand with large crews. The plants are placed into the soil on prepared listed beds that are on 40-inch centers (see Figure 2). The depth of the planting is generally 3 inches, but depends on the size of the transplant plug. Each plant is separated approximately eighteen inches apart going down the seed-line and each parallel seed-line on a single bed is twelve inches apart. The successive plantings of the second, third, and fourth male follow the female planting at approximately ten day intervals. An illustrative planting schedule is as follows:

October 15	female transplanting date
October 15	first male transplanting date
October 25	second male transplanting date
November 4	third male transplanting date
November 14	fourth male transplanting date

The dates are not fixed, but are an approximation for illustrative and non-limiting purposes.

Once all the plantings are accomplished, the field is watched for typical cultural problems found in all broccoli production, whether

for seed or vegetable. These problems include weeds, diseases, insect pests, irrigation, fertilization, and cultivation.

The singular difference for a seed production field as compared to a broccoli production field is the use of rogueing. Rogueing is simply the walking through and examination of a field and checking each plant for correctness to type. Any plant that does not fit the proper description for type is pulled and destroyed or "rogued." The rogueing starts within thirty days of the last male transplanted and continues until the field is at a market ready point, which is generally 100 days. Once the field is at market ready (market ready being the point where the heads are harvestable as a vegetable for sale) the seed production starts. Market ready heads are generally seen in the female and first male in late April to early May of the year following transplanting. The fully developed heads age and then bolt, which is the extension of individual flower stalks. The nick or timing between the male plants bolting and female plants bolting is now the crucial item watched. The female will only set hybrid seed if pollen is in constant and abundant supply from a male plant. The heads of both the male and female plant can be trimmed to accelerate or slow down the flowering to insure abundant male flowers are available as the female plant flowers. Pollen transfer from the male to the female is done by honeybees, which are commercially supplied. Each acre of seed production requires three to five hives of honeybees. The flowering stage will last sixty to eighty days.

The flowering period is followed by the maturation of the seed within seedpods. The maturation period of 40 to 60 days is checked by monitoring the seed development, as it goes from green and water filled, to the dough stage ending with the seed turning from green to brown in color. A judgment call is made, measuring the number of the mature seeds versus seeds yet completed. When the

majority of the seed is mature the female plants are cut by hand and laid in rows (windrowed) to dry down for combining. Ten to twenty days are needed for the plants to dry down.

Combining is a process, which entails the use of a large harvest machine that lifts the broccoli plants from the ground and grinds them for seed preparation. The plant material is cleaned away from the seed by screens and air, leaving only seed. Combining is the initiation of the seed re-conditioning process. Once combined or harvested, the seed is sent to a mill, which further cleans the seed, separates the clean seed by size and weight within a size. All testing for purity, disease, germination, and percent hybridity is done on the clean, sized, and weighted seed. If the seed passes the testing it is canned for sale.

The above method describes the seed production methods for the specific hybrids H7007, H7009, H7022, H2061, H2088, H7021R and H7028 and generally is the method used for all other hybrid broccoli seed production. Hybrid seeds H7007, H7009 H7022, H2061, H2088, H7021R and H70028 were produced by crossing corresponding male lines with 393-2-19 as follows:

H7007 = 393-2-19 X male 7007

H7009 = 393-2-19 X male 7009

H7022 = 393-2-19 X male 5580-2 (same derivation as 7770)

H2061 = 393-2-19 X male 1551 (same derivation as 7881)

H2088 = 393-2-19 male 7009

H7021R = 5580 (same derivation as 7770) X 393-2-19

H7028 = 393-2-19 X male 7028

COMPARATIVE STUDIES

Several studies have been performed to compare and contrast the broccoli lines of this invention with commercially available broccoli lines.

Comparative Analysis Study #1

In study #1, the following broccoli lines were analyzed: Hybrid 7007, hybrid 7008, hybrid 7022, hybrid 7028, male 7007, male 7009, male 7022, male 7028, hybrid 393-2-19, hybrid 393-2-47, Marathon and Pinnacle. Marathon and Pinnacle are commercially available broccoli hybrids. Hybrid 7022 resulted from a cross between 393-12-19 and 5580-2 (393-2-19/5580-2). As indicated above, 5580 is the same derivative as 7770.

Broccoli seeds were sown in the greenhouse. Broccoli seedlings were transplanted to the field on August 8. Daily high and low temperature measurements during the course of study #1 are presented in Table 1. Note that the growing temperatures for study #1 were generally quite warm.

In study #1, the days from direct seeding to 50% harvest; days from transplanting to 50% harvest and the length of the harvest period are shown in Table 2.1. The results indicate that the broccoli lines of this invention have a significantly longer harvest period than the commercially available hybrids Marathon and Pinnacle. A longer period in which the head remains available for harvest offers growers greater flexibility in harvesting and therefore greatly reduces costs. The harvest "holding" ability is due, in part, to heat tolerance.

Table 2.2 shows data summarizing various characteristics of the broccoli plants at harvest. Tables 2.3A and 2.3B show data regarding the characteristics of the outer leaves at harvest. The data indicate that both Pinnacle and Marathon were gray-green in foliage color, which is demonstratively different and less commercially acceptable than the blue green foliage of the heat tolerant lines of the invention.

Table 2.4A-2.4D show characteristics of the broccoli heads at market maturity. Table 2.5 shows flower color.

Table 2.6 shows resistance to various environmental conditions, undesirable characteristics of broccoli and diseases. Of particular importance is that the commercially available varieties Marathon and Pinnacle are much more susceptible to downy mildew virus as compared to the broccoli lines of the invention.

Table 2.7 shows heat tolerance data. Of particular relevance is the low heat tolerance of the commercially available varieties Marathon and Pinnacle as compared to the broccoli lines of this invention.

TABLE 1

Temperature Data for Study #1

Date	Temperature (°F)		
	Max	min	Average
07/03	79	47	62
07/04	84	48	63
07/05	75	52	60
07/06	75	52	62
07/07	75	52	61
07/08	71	48	59
07/09	63	54	58
07/10	70	54	59
07/11	73	53	59
07/12	74	52	59
07/13	85	53	65
07/14	87	54	69
07/15	82	50	63
07/16	72	32	62
07/17	76	56	64
07/18	83	58	68
07/19	89	52	69
07/20	83	53	67
07/21	88	53	71
07/22	100	55	78
07/23	99	59	77
07/24	88	56	69
07/25	95	54	72
07/26	81	58	70
07/27	76	55	63
07/28	78	55	62
07/29	75	56	62
07/30	72	56	61
07/31	72	57	62
08/01	82	57	65
08/02	83	56	65
08/03	88	54	68
08/04	83	56	66
08/05	77	56	64
08/06	74	58	64
08/07	79	59	66
08/08	90	56	71
08/09	98	59	74
08/10	109	60	81
08/11	100	61	78
08/12	91	58	70

TABLE 1

Temperature Data for Study #1

Date	Temperature (°F)		
	Max	min	Average
08/13	83	56	66
08/14	84	52	64
08/15	81	50	62
08/16	86	50	66
08/17	92	53	71
08/18	98	58	75
08/19	97	60	75
08/20	92	57	71
08/21	89	58	68
08/22	74	54	61
08/23	74	53	61
08/24	72	51	61
08/25	69	53	60
08/26	71	52	60
08/27	77	54	63
08/28	81	50	63
08/29	86	51	67
08/30	87	50	67
08/31	83	51	64
09/01	69	54	59
09/02	81	51	61
09/03	91	48	64
09/04	95	52	71
09/05	94	46	72
09/06	95	57	71
09/07	91	55	69
09/08	93	54	71
09/09	98	54	69
09/10	91	58	71
09/11	83	61	69
09/12	89	64	73
09/13	95	68	77
09/14	93	64	75
09/15	77	56	64
09/16	68	56	60
09/17	83	32	70
09/18	85	52	67
09/19	87	55	69
09/20	88	57	68
09/21	76	55	62
09/22	73	53	61

Table 2: Comparative Analysis (Study #1)

2.1. Region of Adaption/Maturity Main Crop at 50% Harvest

#	I.D.	Region of Adaption	Days from Direct Seeding to 50% Harvest	Days from Transplanting to 50% Harvest	Length of Harvest Period in Days
1	Hybrid 7007	Most regions	137	84	7
2	Hybrid 7008	Most regions	137	87	6
3	Hybrid 7022	Most regions	127	77	6
4	Hybrid 7028	Most regions	136	86	6
5	Male 7007	Southwest	135	85	3
6	Male 7009	Southwest	135	85	4
7	Male 7022	Southwest	123	73	5
8	Male 7028	Southwest	138	88	5
9	Hybrid 393-2-19	Most regions	137	87	8
10	Hybrid 393-2-47	Most regions	133	83	6
11	Marathon	Most regions	134	84	4
12	Pinnacle	Southwest	123	73	2

2.2. Study #1
Plant (At Harvest)

#	I.D.	Plant Height (cm)	Head Height (cm)	Plant Branches	Plant Habit	Market Class	Lifecycle	Variety Type
1	Hybrid 7007	76.5	57.5	Few	Intermediate	Fresh Market/ Processing	Annual	First generation hybrid
2	Hybrid 7008	-	-	-	-	-	-	-
3	Hybrid 7022	72	51.5	Few	Spreading	Fresh Market/ Processing	Annual	First generation hybrid
4	Hybrid 7028	82.5	57.5	Few	Intermediate	Fresh Market/ Processing	Annual	First generation hybrid
5	Male 7007	92	65	Few	Intermediate	Fresh Market/ Processing	Annual	Inbred
6	Male 7009	92	76	Few	Intermediate	Fresh Market/ Processing	Annual	Inbred
7	Male 7022	58	35	Few	Compact	Fresh Market/ Processing	Annual	Inbred
8	Male 7028	74.5	51	Few	Intermediate	Fresh Market/ Processing	Annual	Inbred

2.2. Study #1
Plant (At Harvest)

#	I.D.	Plant Height (cm)	Head Height (cm)	Plant Branches	Plant Habit	Market Class	Lifecycle	Variety Type
9	Inbred 393-2-19	62	45.5	Few	Intermediate	Fresh Market/ Processing	Annual	Inbred
10	Inbred 393-2-47	60	48.5	Few	Intermediate	Fresh Market/ Processing	Annual	Inbred
11	Marathon	86.5	56.5	Medium	Spreading	Fresh Market/ Processing	Annual	First generation hybrid
12	Pinnacle	88.5	61.5	Few	Intermediate	Fresh Market/ Processing	Annual	First generation hybrid

2.3A. Study #1
Outer Leaves (At Harvest)

#	I.D.	# Leaves Per Plant	Leaf Width (cm)	Leaf Length (cm)	Petiole Length (cm)	Leaf Attachment	Wax Presence	Foliage Color
1	Hybrid 7007	29	20	52.5	22	Petiolate	Strong	Blue-green
2	Hybrid 7008	-	-	-	-	-	-	-
3	Hybrid 7022	18	14	41	17.5	Petiolate	Strong	Blue-green
4	Hybrid 7028	25	17.5	53.5	19.5	Petiolate	Strong	Blue-green
5	Male 7007	30	16.5	40.5	13.5	Petiolate	Strong	Blue-green
6	Male 7009	26	15.5	47	18	Petiolate	Strong	Blue-green
7	Male 7022	21	23.5	48	17.5	Petiolate	Strong	Blue-green
8	Male 7028	34	15.5	42.5	19.	Petiolate	Strong	Blue-green
9	Inbred 393-2-19	23	14	36	11.5	Petiolate	Strong	Blue-green
10	Inbred 393-2-47	24	16.5	40	17.5	Petiolate	Strong	Blue-green
11	Marathon	50	15.5	50	22	Petiolate	Intermediate	Grey-green
12	Pinnacle	27	16	46.5	21	Petiolate	Intermediate	Grey-green

2.3B. Study #1
Outer Leaves (At Harvest)

#	I.D.	Leaf Shape	Leaf Base	Leaf Apex	Leaf Margins	Leaf Veins	Midrib	Blistering	Attitude	Leaf tip Torsion	Upper Side of Leaf Profile
1	Hybrid 7007	Elliptic	Blunt	Blunt	Slightly wavy	Inter-mediate	Slightly raised	None	Semi-erect	Weak	Concave
2	Hybrid 7008	-	-	-	-	-	-	-	-	-	-
3	Hybrid 7022	Narrow elliptic	Blunt	Blunt	Slightly wavy	Inter-mediate	Slightly raised	None	Semi-erect	None	Planar
4	Hybrid 7028	Elliptic	Blunt	Blunt	Slightly wavy	Inter-mediate	Slightly raised	None	Semi-erect	None	Concave
5	Male 7007	Elliptic	Blunt	Blunt	Slightly wavy	Inter-mediate	Slightly raised	None	Erect	Weak	Concave
6	Male 7009	Elliptic	Pointed	Blunt	Slightly wavy	Inter-mediate	Slightly raised	None	Erect	Intermediate	Planar
7	Male 7022	Broad elliptic	Blunt	Blunt	Slightly wavy	Inter-mediate	Not raised	None	Horizontal/ Semi-erect	Weak	Concave

2.3B. Study #1

Outer Leaves (At Harvest)

#	I.D.	Leaf Shape	Leaf Base	Leaf Apex	Leaf Margins	Leaf Veins	Midrib	Blistering	Attitude	Leaf tip Torsion	Upper Side of Leaf Profile
8	Male 7028	Elliptic	Blunt	Blunt	Slightly wavy	Inter-mediate	Slightly raised	None	Erect	None	Concave
9	Inbred 393-2-19	Elliptic	Blunt	Blunt	Slightly wavy	Inter-mediate	Slightly raised	None	Erect	None	Concave
10	Inbred 393-2-47	Elliptic	Blunt	Blunt	Slightly wavy	Inter-mediate	Slightly raised	None	Semi-erect	None	Concave
11	Marathon	Narrow elliptic	Blunt	Blunt	Very wavy	Inter-mediate	Slightly raised	None	Horizontal/ Semi-erect	None	Concave
12	Pinnacle	Elliptic	Blunt	Blunt	Slightly wavy	Inter-mediate	Slightly raised	None	Horizontal	None	Concave

2.4A. Study #1

Head (At Market Maturity)

#	I.D.	Head Diameter (cm)	Head Depth (cm)	Head Weight (gm)	Head Color	Head Shape
1	Hybrid 7007	17	13.5	446.3	Blue/Green	Transverse broad elliptic
2	Hybrid 7008	-	-	352	-	-
3	Hybrid 7022	15	10.5	377.2	Blue/Green	Transverse narrow elliptic
4	Hybrid 7028	15	11.5	364.4	Blue/Green	Transverse broad elliptic
5	Male 7007	10	8	93.7	Blue/Green	Circular
6	Male 7009	10	8.5	126.2	Blue/Green	Transverse broad elliptic
7	Male 7022	15	10	289.5	Blue/Green	Transverse narrow elliptic
8	Male 7028	11	9.5	165.9	Blue/Green	Circular
9	Inbred 393-2-19	13	10	325.7	Blue/Green	Transverse elliptic
10	Inbred 393-2-47	11.5	9	194.7	Blue/purple	Transverse broad elliptic
11	Marathon	14.5	12.5	300	Medium green	Transverse elliptic
12	Pinnacle	14	9.5	274.6	Medium green	Transverse elliptic
13	Male 7008	-	-	150	-	-

2.4B. Study #1
Head (At Market Maturity)

#	I.D.	Dome Shape	Head Size	Compactness	Surface Knobbling	Bead Size	Flower Buds
1	Hybrid 7007	Semi-domed	Large	Short pedicels (tight)	Fine	Medium	Even in size
2	Hybrid 7008	-	-	-	-	-	-
3	Hybrid 7022	Very Deeply domed	Large	Short pedicels (tight)	Fine	Medium	Even in size
4	Hybrid 7028	Semi-domed	Medium	Short pedicels (tight)	Fine	Medium	Even in size
5	Male 7007	Domed	Small	Short pedicels (tight)	Fine	Large	Even in size
6	Male 7009	Semi-domed	Small	Medium pedicels	Fine	Large	Even in size
7	Male 7022	Very deeply domed	Large	Short pedicels (tight)	Fine	Medium	Even in size
8	Male 7028	Domed	Small	Medium pedicels	Fine	Medium	Even in size
9	Inbred 393-2-19	Deep domed	Medium	Short pedicels (tight)	Fine	Small	Even in size
10	Inbred 393-2-47	Semi-domed	Medium	Short pedicels (tight)	Fine	Medium	Even in size
11	Marathon	Deep Domed	Medium	Short pedicels (tight)	Medium	Small	Even in size
12	Pinnacle	Deep Domed	Medium	Medium pedicels	Large	Small	Uneven in size

2.4C. Study #1

Head (At Market Maturity) Anthocyanin Coloration

#	I.D.	Leaf Axils	Leaf Veins	Leaf Blade	Entire Plant	Leaf Petiole
1	Hybrid 7007	Absent	Absent	Absent	Absent	Absent
2	Hybrid 7008	-	-	-	-	-
3	Hybrid 7022	Absent	Absent	Absent	Absent	Absent
4	Hybrid 7028	Absent	Absent	Absent	Absent	Absent
5	Male 7007	Absent	Absent	Absent	Absent	Absent
6	Male 7009	Absent	Absent	Absent	Absent	Absent
7	Male 7022	Absent	Absent	Absent	Absent	Absent
8	Male 7028	Absent	Absent	Absent	Absent	Absent
9	Inbred 393-2-19	Absent	Absent	Absent	Absent	Absent
10	Inbred 393-2-47	Slight Pressure	Absent	Absent	Absent	Absent
11	Marathon	Absent	Absent	Absent	Absent	Absent
12	Pinnacle	Absent	Absent	Absent	Absent	Absent

2.4D. Study #1
Head (At Market Maturity)

#	I.D.	Color of Head Leaves	Secondary Heads	Prominence of Secondary Heads	Number of Secondary Heads
1	Hybrid 7007	Green	Completely Absent	Weak	0
2	Hybrid 7008	-	-	-	-
3	Hybrid 7022	Green	Completely Absent	Weak	0
4	Hybrid 7028	Green	Basal	Weak	3
5	Male 7007	Green	Completely Absent	Weak	0
6	Male 7009	Green	Basal	Weak	4
7	Male 7022	Green	Completely Absent	Weak	0
8	Male 7028	Green	Completely Absent	Weak	0
9	Inbred 393-2-19	Green	Completely Absent	Weak	0
10	Inbred 393-2-47	Green	Completely Absent	Weak	0
11	Marathon	Green	Auxiliary along entire main stem up to main head	Weak	3
12	Pinnacle	Green	Basal	Weak	1

2.5. Study #1
Flower Color

#	I.D.	Flower Color	Flower Stalk Color
1	Hybrid 7007	Yellow	Green
2	Hybrid 7008	-	-
3	Hybrid 7022	Yellow	Green
4	Hybrid 7028	Yellow	-
5	Male 7007	Yellow	-
6	Male 7009	Yellow	-
7	Male 7022	Yellow	Green
8	Male 7028	Yellow	-
9	Inbred 393-2-19	Yellow	Green
10	Inbred 393-2-47	Yellow	Green
11	Marathon	Yellow	Green
12	Pinnacle	Yellow	Green

2.6. Study #1
Resistance*

#	I.D.	Downey Mildew	Buttoning	Blindness	Bolting	Brown Beads	Drought	Cold	Hollow stem	Riceyness	Whiptail
1	Hybrid 7007	9	8	8	5	9	7	5	8	9	9
2	Hybrid 7008	-	-	-	-	-	-	-	-	-	-
3	Hybrid 7022	9	8	8	4	8	7	3	8	9	9
4	Hybrid 7028	9	8	8	4	9	7	4	8	9	9
5	Male 7007	9	8	8	3	9	8	3	9	9	9
6	Male 7009	9	8	8	5	9	8	3	9	9	9
7	Male 7022	-	8	8	6	8	8	3	8	9	9
8	Male 7028	9	8	8	5	9	8	3	8	9	9
9	Inbred 393-2-19	9	8	8	6	9	8	6	9	9	9
10	Inbred 393-2-47	9	8	8	5	9	8	5	9	9	9
11	Marathon	3	8	8	7	8	7	7	8	9	9
12	Pinnacle	3	8	8	6	5	5	4	8	5	9

* 1 = Most susceptible
5 = Intermediate
9 = Most resistant

2.7. Study #1
Heat Tolerance*

#	I.D.	Heat Tolerance*
1	Hybrid 7007	9
2	Hybrid 7008	-
3	Hybrid 7022	8
4	Hybrid 7028	9
5	Male 7007	9
6	Male 7009	8
7	Male 7022	7
8	Male 7028	8
9	Inbred 393-2-19	8
10	Inbred 393-2-47	8
11	Marathon	2
12	Pinnacle	4

* 1 = Most susceptible
5 = Intermediate
9 = Most tolerant

Comparative Analysis Study #2

In a second study (Study #2) various broccoli lines were analyzed and characterized for heat tolerance. Daily high and low temperature measurements for study #2 are presented in Table 3. As in study #2, the daily temperatures were generally quite warm and on some days hot.

In study #2, the following broccoli lines were analyzed: Hybrid 7007, Hybrid 7009, Hybrid 7028, Male 7007, Male 7009, Male 7028, Inbred 393-2-19, Inbred 393-2-47, Marathon, Pinnacle, 98-2061, 98-2088, Inbred 393-2-32 and 4267-1. 98-2061 results from a cross between 393-2-19 and 1551 (393-2-19/1551). As indicated above, 1551 is the same derivative as 7881. The line 98-2088 results from across between 393-2-19 and M7009 (393-2-19/1551). Line 2192 is derived from the same line as 4267-1.

In study #2, broccoli seeds were sown in the greenhouse on April 27. Broccoli seedlings were transferred to the field on June 13.

The comparative data collected in study #2 are shown in Table 4.

Table 4.1 shows the length of the harvest period, the plant and head height at harvest, the type of plant branches and the plant habit at harvest. Of particular relevance is that the broccoli plants of this invention have a significantly longer harvest period than the commercially available hybrids Marathon and Pinnacle. A longer harvest period offers growers greater flexibility in harvesting and therefore greatly reduces costs.

Tables 4.2A-4.2C show characteristics of outer leaves at harvest. Tables 4.3A-4.3B and 4.4A show characteristics of the harvested broccoli heads. Table 4.5 shows heat tolerance data.

Of particular relevance is the data in Table 4.5, which shows

that the broccoli plants of this invention are heat tolerant whereas the commercially available varieties are not.

TABLE 3

Temperature Data for Study #2

Date	Temperature (°F)		
	Max	min	Average
08/08	75	57	63
08/09	81	60	66
08/10	77	58	65
08/11	78	57	65
08/12	79	53	63
08/13	78	55	63
08/14	83	54	64
08/15	77	56	62
08/16	73	56	63
08/17	89	53	63
08/18	83	54	66
08/19	82	59	69
08/20	77	59	66
08/21	87	59	69
08/22	85	56	70
08/23	83	59	69
08/24	82	62	70
08/25	83	57	68
08/26	83	57	68
08/27	83	51	70
08/28	83	56	69
08/29	84	59	69
08/30	82	55	67
08/31	83	59	70
09/01	84	59	70
09/02	81	56	68
09/03	85	59	69
09/04	95	59	73
09/05	87	58	70
09/06	80	55	65
09/07	88	53	66
09/08	86	59	69
09/09	82	55	66
09/10	80	54	66
09/11	79	58	67
09/12	78	54	65
09/13	78	53	63
09/14	79	54	65
09/15	80	56	66

TABLE 3

Temperature Data for Study #2

Date	Temperature (°F)		
	Max	min	Average
09/16	85	51	68
09/17	79	54	66
09/18	78	54	64
09/19	82	48	63
09/20	88	51	68
09/21	89	51	66
09/22	87	49	66
09/23	102	54	75
09/24	97	59	74
09/25	87	61	72
09/26	80	57	67
09/27	87	52	68
09/28	95	52	73
09/29	90	59	69
09/30	94	54	65
10/01	74	56	63
10/02	76	57	64
10/03	83	51	66
10/04	81	51	65
10/05	83	50	64
10/06	69	49	59
10/07	70	46	57
10/08	72	43	57
10/09	65	52	60
10/10	62	44	53
10/11	68	44	55
10/12	74	44	57
10/13	83	42	61
10/14	89	44	65
10/15	95	49	68
10/16	96	50	68
10/17	87	50	64
10/18	81	46	59
10/19	64	49	56
10/20	70	49	57
10/21	74	45	55
10/22	70	45	54
10/23	68	49	57
10/24	72	42	55
10/25	75	38	56

TABLE 3

Temperature Data for Study #2

Date	Temperature (°F)		
	Max	min	Average
10/26	79	40	57
10/27	75	42	56

TABLE 4 COMPARATIVE ANALYSIS Study #2

4.1. Maturity: Main Crop at 50% Harvest/Plant At Harvest

#	I.D.	Length of Harvest Period (Days)	Plant Height (inches)	Head Height (inches)	Plant Branches	Plant Habit
1	Hybrid 7007	5	30	22	Medium	Spreading
2	Hybrid 7009	5	28¼	22	Medium	Spreading
3	Hybrid 7028	4	25 5/8	17	Medium	Spreading
4	Male 7007	3	36	33	Medium	Spreading
5	Male 7009	4	37	25½	Many	Spreading
6	Male 7028	-	31	23½	Medium	Spreading
7	Inbred 393-2-19	6	27	20	Medium	Spreading
8	Inbred 393-2-47	4.5	26	20	Few	Spreading
9	Marathon	1	30.5	20	Many	Intermediate
10	Pinnacle	1	30	26¾	Medium	Intermediate
11	98-2061	5	26	18	Medium	Intermediate
12	98-2088	4	29¼	23¾	Medium	Intermediate
13	Inbred 393-2-32	6	27	18¼	Medium	Intermediate
14	98-2192	6	28	16	Medium	Spreading

4.2A. Study #2
Outer Leaves (At Harvest)

#	I.D.	# Leaves Per Plant	Leaf Width (inches)	Leaf Length (inches)	Petiole Length (inches)	Length/Width Ratio
1	Hybrid 7007	23	8	18¼	5	2:1
2	Hybrid 7009	26	8	17	5	2:1
3	Hybrid 7028	22	6½	16	4¾	2:1
4	Male 7007	24	10	19	7	2:1
5	Male 7009	32	9	21½	6¾	2:1
6	Male 7028	18	10½	22½	6½	2:1
7	Inbred 393-2-19	21	7	16½	5½	2:1
8	Inbred 393-2-47	17	5¾	11¾	3½	2:1
9	Marathon	32	7	18	8¼	2:1
10	Pinnacle	25	5 5/8	14½	7¼	2:1
11	98-2061	19	6½	16½	6¼	2:1
12	98-2088	28	7¼	14¾	3½	2:1
13	Inbred 393-2-32	21	6½	19	8¼	2:1
14	98-2192	28	8¼	18½	7¾	2:1

4.2B. Study #2

Outer Leaves (At Harvest)

#	I.D.	Leaf Attachment	Wax Presence	Foliage Color	Leaf Shape	Leaf Base
1	Hybrid 7007	Petiolate	Strong	Medium green	Elliptic	Pointed
2	Hybrid 7009	Petiolate	Strong	Medium green	Elliptic	Blunt
3	Hybrid 7028	Petiolate	Strong	Medium green	Elliptic	Blunt
4	Male 7007	Petiolate	Strong	Medium green	Elliptic	Blunt
5	Male 7009	Petiolate	Strong	Medium green	Broad elliptic	Blunt/pointed
6	Male 7028	Petiolate	Strong	Medium green	Broad elliptic	Blunt/pointed
7	Inbred 393-2-19	Petiolate	Strong	Medium green	Elliptic	Blunt
8	Inbred 393-2-47	Petiolate	Strong	Medium green	Elliptic	Blunt
9	Marathon	Petiolate	Strong	Blue-green	Elliptic	Blunt
10	Pinnacle	Petiolate	Strong	Medium green	Narrow elliptic	Blunt
11	98-2061	Petiolate	Strong	Dark green	Elliptic	Blunt
12	98-2088	Petiolate	Strong	Medium green	Narrow elliptic	Blunt
13	Inbred 393-2-32	Petiolate	Strong	Medium green	Elliptic	Blunt
14	98-2192	Petiolate	Strong	Dark green	Elliptic	Blunt

4.2C. Study #2

Outer Leaves (At Harvest)

#	I.D.	Leaf Apex	Leaf Margins	Leaf Veins	Attitude	Torsion	Profile
1	Hybrid 7007	Blunt	Slightly wavy	Intermediate	Horizontal	Weak	Planar
2	Hybrid 7009	Blunt	Slightly wavy	Intermediate	Semi-erect/erect	Weak	Planar
3	Hybrid 7028	Blunt	Slightly wavy	Thin	Semi-erect/erect	Weak	Planar
4	Male 7007	Blunt	Slightly wavy	Intermediate	Semi-erect/erect	Weak	Planar
5	Male 7009	Blunt	Very wavy	Intermediate	Semi-erect/erect	Weak	Planar/convex
6	Male 7028	Blunt	Slightly wavy	Thick	Horizontal/semi-erect	Weak	Planar
7	Inbred 393-2-19	Blunt	Slightly wavy	Intermediate	Semi-erect	Weak	Concave
8	Inbred 393-2-47	Blunt	Slightly wavy	Intermediate	Horizontal/semi-erect	Weak	Concave/planar
9	Marathon	Blunt	Very wavy	Intermediate	Horizontal	Intermediate	Concave
10	Pinnacle	Blunt	Slightly wavy	Intermediate	Semi-erect/erect	Intermediate	Convex
11	98-2061	Blunt	Slightly wavy	Thin	Semi-erect	Weak	Planar
12	98-2088	Blunt	Slightly wavy	Intermediate	Semi-erect/erect	Weak	Concave
13	Inbred 393-2-32	Blunt	Slightly wavy	Intermediate	Horizontal	Weak	Planar
14	98-2192	Blunt	Slightly wavy	Intermediate	Semi-erect/erect	Weak	Planar/convex

4.3A. Study #2

Head (At Market Maturity)

#	I.D.	Head Diameter (inches)	Head Depth (inches)	Head Weight (gm)	Color	Head Shape
1	Hybrid 7007	10	5 ¼	904.9	Blue/green	Transverse elliptic
2	Hybrid 7009	7	3 ¼	306.4	Purple/ blue/green	Transverse elliptic
3	Hybrid 7028	4 ¾	3	117	Blue/green	Transverse elliptic
4	Male 7007	4 ¼	2 ½	85.6	Medium green	Circular
5	Male 7009	3 ¾	2 ¼	103.6	Dark green/ Blue/green	Transverse broad elliptic
6	Male 7028	6	3 ½	450.3	Light purple/ dark green	Transverse elliptic
7	Inbred 393-2-19	5	3	176.3	Blue/green purple	Transverse elliptic narrow
8	Inbred 393-2-47	4 ¾	2 ¾	136.4	Light green/ purple	Transverse elliptic
9	Marathon	4 ½	2 ¼	313.1	Yellow	Transverse elliptic
10	Pinnacle	6 ¼	4 ¾	336.3	Blue/green	Transverse elliptic
11	98-2061	5 ½	3	184.1	Blue/green	Transverse broad elliptic
12	98-2088	5 ¾	3 ½	184.1	Blue/green/ purple	Transverse elliptic
13	Inbred 393-2-32	3 ¼	2 ½	67.2	Medium green/ blue/green	Transverse broad elliptic
14	98-2192	5 ½	3	226.0	Blue/green	Transverse elliptic

4.3B. Study #2

Head (At Market Maturity)

#	I.D.	Dome Shape	Head Size	Compactness	Surface Knobbling	Beads Size	Flower Buds
1	Hybrid 7007	Domed	Large	Medium pedicels	Medium	Medium/large	Even in size
2	Hybrid 7009	Deep-domed	Medium	Medium pedicels	Medium	Medium	Even in size
3	Hybrid 7028	Semi-domed	Medium	Short pedicels	Medium	Small	Even in size
4	Male 7007	Domed	Small	Medium pedicels	Fine	Medium	Even in size
5	Male 7009	Deep-domed	Small	Short pedicels	Fine	Small	Even in size
6	Male 7028	Deep-domed	Medium	Short pedicels	Medium	Small	Even in size
7	Inbred 393-2-19	Deep-domed	Medium	Short pedicels	Medium	Small	Even in size
8	Inbred 393-2-47	Domed	Medium	Short pedicels	Medium	Small	Even in size
9	Marathon	Domed	Small	Short pedicels	Medium	Small	Uneven in size
10	Pinnacle	Domed	Medium	Long pedicels	Coarse	Large	Uneven in size
11	98-2061	Semi-domed	Medium	Short pedicels	Medium	Small	Even in size
12	98-2088	Semi-domed	Medium	Medium pedicels	Medium	Medium	Even in size
13	Inbred 393-2-32	Semi-domed	Small	Short pedicels	Fine	Small	Even in size
14	98-2192	Deep-domed	Medium	Short pedicels	Medium	Small	Even in size

4.4A. Study #2
Head (At Market Maturity)

#	I.D.	Color of Head Leaves	Secondary Heads	Prominence of Secondary Heads	# of Secondary Heads
1	Hybrid 7007	---	---	---	---
2	Hybrid 7009	---	---	---	---
3	Hybrid 7028	---	Basal	Weak	1
4	Male 7007	---	Basal	Weak	0
5	Male 7009	Green	---	---	---
6	Male 7028	---	---	---	---
7	Inbred 393-2-19	---	---	---	---
8	Inbred 393-2-47	---	---	---	---
9	Marathon	---	Completely absent	Weak	0
10	Pinnacle	---	Basal	Weak	3
11	98-2061		Basal	Weak	1
12	98-2088		Basal	Intermediate	4
13	Inbred 393-2-32		---	---	---
14	98-2192		Combination	Intermediate	3

4.5. Study #2
Heat Tolerance

#	I.D.	Heat Tolerance*
1	Hybrid 7007	7
2	Hybrid 7009	8
3	Hybrid 7028	7
4	Male 7007	---
5	Male 7009	7/8
6	Male 7028	---
7	Inbred 393-2-19	5/6
8	Inbred 393-2-47	5/6
9	Marathon	2
10	Pinnacle	1
11	98-2061	7/8
12	98-2088	7
13	Inbred 393-2-32	---
14	98-2192	8/9

* 1 = Most susceptible
5 = Intermediate
9 = Most tolerant

Comparative Analysis Study #3

In a third study (Study #3) various broccoli lines were analyzed and characterized for heat tolerance. Lines tested included H7009, H7007, H7028, H7010 H7021R, Marathon, Pinnacle, etc. Lines which include a backslash (/) between the two lines represent a cross between the two lines. The second line on the right side of the backslash, is the "male" line in the cross. The "male" line in the cross is the source of the pollen in the cross. For example 393-2-19/7770 represents a cross between 393-2-19 and 7770 wherein 7770 was the source of the pollen and 393-2-19 was the recipient of the pollen. Single plant selections were made of the crosses. The resulting seed was then selfed. The data presented is summary data based upon an entire row of plants.

As indicated above, H7021R results from a cross between 5580 and 393-2-19 (5580/393-2-19)

Daily high and low temperature measurements for study #3 are presented in Table 5. As in studies #'s 1 and 2, the growth temperatures during study #3 were generally quite warm and sometimes hot.

Various broccoli lines were analyzed for heat tolerance. The heat tolerance data is presented in Table 6.

The commercial hybrids (Marathon, Pinnacle, Premium Crop, Patriot, Laguna, Monte Cristo, Greenbelt, Everest, CMS Liberty, and Landmark) averaged a score of 2.83 for heat tolerance. The new heat tolerant hybrids (7007, 7009, and 7028) that are the subject of this patent application averaged 7.00 for heat tolerance. As discussed above, the heat tolerance scale goes from one (1) to nine (9), with one (1) the most susceptible and nine (9) very highly resistant as described above. In general, ratings of five (5) or below are unmarketable in a heat stress growth condition and represent

significant economic loss to the broccoli growers if such a level of heat stress reaction occurs in their broccoli fields.

The broccoli lines from study #3 were also comparatively analyzed for bead size, yield, head shape, extension and maturity. The results are presented in Table 7.

The bead size rating is on a scale of 1-5. A bead size rating of 5.0 represents very, very large beads. A bead size rating of 1.0 represents very, very small beads such as cauliflower beads. A bead size rating of 4.0 represents large beads. An ideal bead size rating is 3.0 to 4.0 with a maximum desirable rating of 3.7 to 3.8. A combination of bead size rating of 3.8 to 4.0 combined with a high bead size uniformity rating is also acceptable.

The yield rating is on a scale of 1 to 10 where a rating of 10 represents a maximum estimated yield for a particular trial. A yield rating of 8.0 compared to a yield rating of 7.0 represents an approximate increase in yield of 50%. While high yields are generally desirable, at the highest yield ratings, hollow core may develop undesirably. A combination of high yield, high heat tolerance (and, therefore head-holding ability) good extension and good uniformity are most desirable. The heat tolerant lines and hybrids of the invention generally exhibited high yields. The high yielding capacity of these lines and hybrids is thought to be due, in part, to an ability to keep increasing head size while maintaining desirable commercial characteristics under heat stress.

The head shape, extension and maturity ratings were on a 0 to 10 scale. Head shape is an important selection criterion for broccoli. Head shape ratings of 7.0 to 8.0 are most desired. A head shape score of 3.0 represents a completely flat to nearly concave head. A head shape score of 4.0 to 5.0 represents a small head not yet approaching a semi-dome. A head shape score of 6.0 represents a semi-dome shaped head. A head shape score of 7.0 represents a

good, solid dome. A head shape score of 8.0 represents a deep dome. A head shape score of 9.0 represents a very pointed dome in the shape of a Christmas tree.

Head extension is also an important selection criterion. Head extension is a comparative measurement of the distance between the broccoli head itself and the leaves surrounding the broccoli. If the head is surrounded by leaves, the head is difficult to harvest. Ideally, the head will be extended up above the leaves to permit easy harvesting of the broccoli. An extension rating of 3.0 represents a head that is buried fairly deep within the leaf canopy. A head extension rating of 5.0 represents a plant having a head which extends only slightly above the leaf canopy. A head extension value of 7.0 represents significant extension of the broccoli head out of the canopy. Commercially available broccoli line Marathon has a head extension rating of 6.5-7.0. The beat tolerant broccoli lines of this invention have an extension rating of generally around 7.3. An extension rating of less than 5.0 is undesirable because the head is surrounded by too many leaves making the broccoli difficult to harvest.

Maturity is also an important selection criterion. The smaller the maturity rating number the earlier the harvest date. The larger the maturity rating number the later the harvest date. A late harvest date is indicative of a line which takes longer to reach maturity and, therefore, longer to produce a commercially acceptable head. Generally, a smaller maturity rating number is preferred because the broccoli grower is able to harvest his/her crop sooner. Later maturing lines (with higher maturity rating numbers) are acceptable so long as they continue to produce commercially acceptable heads with a proper head size, coloring, head shape, etc.

Prior art commercially available line Marathon generally has a maturity rating of 5.5/6.0. In contrast, the heat tolerant broccoli lines of this invention generally have a maturity rating of around 6.2.

TABLE 5

Temperature Data for Study #3

Date	Temperature (°F)		
	Max	Min	Average
07/03	79	47	62
07/04	84	48	63
07/05	75	52	60
07/06	75	52	62
07/07	75	52	61
07/08	71	48	59
07/09	63	54	58
07/10	70	54	59
07/11	73	53	59
07/12	74	52	59
07/13	85	53	65
07/14	87	54	69
07/15	82	50	63
07/16	72	32	62
07/17	76	56	64
07/18	83	58	68
07/19	89	52	69
07/20	83	53	67
07/21	88	53	71
07/22	100	55	78
07/23	99	59	77
07/24	88	56	69
07/25	95	54	72
07/26	81	58	70
07/27	76	55	63
07/28	78	55	62
07/29	75	56	62
07/30	72	56	61
07/31	72	57	62
08/01	82	57	65
08/02	83	56	65
08/03	88	54	68
08/04	83	56	66
08/05	77	56	64
08/06	74	58	64
08/07	79	59	66
08/08	90	56	71

TABLE 5

Temperature Data for Study #3

Date	Temperature (°F)		
	Max	Min	Average
08/09	98	59	74
08/10	109	60	81
08/11	100	61	78
08/12	91	58	70
08/13	83	56	66
08/14	84	52	64
08/15	81	50	62
08/16	86	50	66
08/17	92	53	71
08/18	98	58	75
08/19	97	60	75
08/20	92	57	71
08/21	89	58	68
08/22	74	54	61
08/23	74	53	61
08/24	72	51	61
08/25	69	53	60
08/26	71	52	60
08/27	77	54	63
08/28	81	50	63
08/29	86	51	67
08/30	87	50	67
08/31	83	51	64
09/01	69	54	59
09/02	81	51	61
09/03	91	48	64
09/04	95	52	71
09/05	94	46	72
09/06	95	57	71
09/07	91	55	69
09/08	93	54	71
09/09	98	54	69
09/10	91	58	71
09/11	83	61	69
09/12	89	64	73
09/13	95	68	77
09/14	93	64	75
09/15	77	56	64

TABLE 5

Temperature Data for Study #3

Date	Temperature (°F)		
	Max	Min	Average
09/16	68	56	60
09/17	83	32	70
09/18	85	52	67
09/19	87	55	69
09/20	88	57	68
09/21	76	55	62
09/22	73	53	61
09/23	72	52	60
09/24	71	51	59
09/25	65	54	59
09/26	65	49	57
09/27	71	46	46
09/28	73	52	60
09/29	64	48	57
09/30	70	56	59
10/01	63	56	58
10/02	69	47	59
10/03	69	44	55
10/04	76	43	58
10/05	83	44	63
10/06	87	47	65
10/07	78	45	60
10/08	74	51	61
10/09	72	43	57
10/10	75	45	57
10/11	78	40	56
10/12	73	46	58
10/13	75	45	59
10/14	69	32	59
10/15	71	40	54
10/16	74	43	57
10/17	77	39	57

TABLE 6

Comparative Analysis: Study #3

#	ID	Heat Tolerance Rating (0-9)**
1	Marathon	1
2	Pinnacle	3
4	H7009	8
5	Premium Crop	4-
6	H7007	7+
8	H7028	6
9	Patriot	1
10	H7010	
11	Laguna	5
12	H7021R	5-
13	Montecristo	4.1
14	Greenbelt	3-
15	393-2-19/7770	5-
16	393-2-19/7778	6-
17	393-2-19/7861	6
18	Sultan	2.5
19	Tierra	6-
20	393-2-19/7864	
21	393-2-19/7865	4+
22	Everest	2
23	Liberty	2+
24	Marathon	3
25	7881/M7007	7-
26	7770-2/393-2-47	6
27	7770/7935	6
28	7770/7935	6
29	7770/7887	7-
30	Landmark	2
31	H7009	7
32	8092/7825	3
33	8092/7795	4+
34	8092/7883	
35	8030/7935	
36	8030/7914	

TABLE 6

Comparative Analysis: Study #3

#	ID	Heat Tolerance Rating (0-9)**
37	H7007	7-
38	Pinnacle	2+
39	Greenbelt	2
40	393-2-19/1692	7-
41	393-2-19/1524	

** 0 = Most susceptible
5 = Intermediate
9 = Most tolerant

TABLE 7

Comparative Analysis: Study #3

#	ID	Bead Size	Yield	Head Shape	Extension	Maturity
1	7770	3.5	7.3	---	---	---
2	7778	3.7	6.1	7.0	7.5	3.7
3	7861	3.7	4.0	7.0	7.0	5.5
4	7864	3.5	6.0	6.7	---	8.0
5	7865	3.6	7.0	6.0	7.2	7.0
6	7881	3.7	7.3	6.7	5.0	7.0
7	7887	3.8	6.7	7.0	7.7	6.7
8	7935	3.7	---	6.7	7.4	5.0
9	8092	3.5	7.7	6.0	5.3	7.0
10	7883	3.8	---	7.3	7.5	6.7
11	7914	3.6	---	5.7	7.6	6.0
12	Pinnacle	3.7	7.0	6.3	7.0	4.0

5

Comparative Analysis Study #4

In a fourth study, various broccoli lines were analyzed and characterized for heat tolerance. Daily high and low temperature measurements for study #3 are presented in Table 8. The maximum daily temperatures during study #4 were generally cooler than the maximum daily temperatures of study #3. In the fourth study, the maximum daily temperature was never greater than 92°F. In contrast, during study #3, the maximum daily temperature was greater than 95°F on several days.

The heat tolerance ratings for several broccoli lines analyzed during study #4 are present in Table 9. The heat tolerant broccoli lines of the invention consistently exhibited heat tolerance ratings of around 7.0. In contrast, the heat tolerant ratings for the commercially available lines for study #4 averaged around 5.0. The heat tolerance ratings for the commercially available lines were generally higher in study #4 than study #3 because the temperatures were cooler during study #4 than in study #3. Since the commercially available lines were exposed to generally cooler temperatures in study #4, the heat tolerance ratings for these lines were higher during study #4.

In study #4, the heat tolerant broccoli lines were also compared to commercially available lines regarding yield, bead size, head shape, extension and uniformity. The rating scale is the same as that for comparative study #3, Table 7. Uniformity represents a comparative measurement of the similarity between the various plants within a line. High uniformity is desired by growers because it allows them to maximize their harvest efficiency. A minimum uniformity rating of 6.0 to 6.5 is generally viewed as commercially acceptable. A rating of 8.0 represents highly uniform broccoli lines. Uniformity values less than 6.0 are generally viewed as commercially unacceptable. The results are presented in Table 10.

In addition to being heat tolerant, the lines of this invention consistently gave higher head shape and extension ratings than the commercially available lines.

TABLE 8

Temperature Data for Study #4

Date	Temperature (°F)	
	Max	Min
05/01	66	50
05/02	65	49
05/03	66	52
05/04	70	41
05/05	83	43
05/06	79	49
05/07	76	51
05/08	75	42
05/09	74	41
05/10	79	42
05/11	74	50
05/12	75	54
05/13	79	42
05/14	68	46
05/15	71	42
05/16	76	42
05/17	82	46
05/18	78	48
05/19	69	51
05/20	67	52
05/21	82	46
05/22	81	49
05/23	75	52
05/24	80	52
05/25	80	53
05/26	75	51
05/27	78	51
05/28	75	51
05/29	61	52
05/30	73	53
05/31	75	51
06/01	70	52
06/02	65	53
06/03	68	48
06/04	68	51
06/05	80	52
06/06	80	53

TABLE 8

Temperature Data for Study #4

Date	Temperature (°F)	
	Max	Min
06/07	75	43
06/08	77	44
06/09	75	44
06/10	81	48
06/11	81	47
06/12	85	51
06/13	84	52
06/14	82	58
06/15	80	57
06/16	84	52
06/17	84	49
06/18	88	51
06/19	84	52
06/20	84	50
06/21	79	54
06/22	86	53
06/23	83	52
06/24	85	54
06/25	83	52
06/26	83	49
06/27	95	49
06/28	99	54
06/29	98	55
06/30	87	51
07/01	87	52
07/02	85	52
07/03	87	52
07/04	87	52
07/05	87	52
07/06	87	52
07/07	87	51
07/08	88	52
07/09	88	52
07/10	90	52
07/11	90	52
07/12	90	53
07/13	91	55
07/14	90	55

TABLE 8

Temperature Data for Study #4

Date	Temperature (°F)	
	Max	Min
07/15	87	53
07/16	86	54
07/17	88	54
07/18	89	54
07/19	89	54
07/20	89	54
07/21	89	53
07/22	89	53
07/23	87	54
07/24	87	54
07/25	89	54
07/26	89	54
07/27	89	53
07/28	89	54
07/29	90	54
07/30	88	54
07/31	90	54
08/01	90	54
08/02	88	54
08/03	88	54
08/04	88	54
08/05	90	53
08/06	91	54
08/07	90	55
08/08	90	54
08/09	90	55
08/10	88	55
08/11	85	55
08/12	86	54
08/13	85	54
08/14	86	54
08/15	87	54
08/16	88	54
08/17	87	54
08/18	86	54
08/19	86	54
08/20	86	53
08/21	85	52

TABLE 8

Temperature Data for Study #4

Date	Temperature (°F)	
	Max	Min
08/22	85	53
08/23	88	53
08/24	87	53
08/25	87	53
08/26	87	54
08/27	88	53
08/28	89	53
08/29	89	53
08/30	86	54
08/31	87	54
09/01	89	53
09/02	88	53
09/03	88	53
09/04	88	54
09/05	88	54
09/06	86	54
09/07	86	54
09/08	86	53
09/09	86	53
09/10	86	53
09/11	86	53
09/12	85	52
09/13	84	53
09/14	85	53
09/15	85	52
09/16	84	52
09/17	84	52
09/18	83	52
09/19	84	51
09/20	84	51
09/21	86	50
09/22	86	50
09/23	85	51
09/24	86	52
09/25	84	53
09/26	82	51
09/27	83	53
09/28	83	51

TABLE 8

Temperature Data for Study #4

Date	Temperature (°F)	
	Max	Min
09/29	84	52
09/30	85	51
10/01	84	50
10/02	82	50
10/03	83	50
10/04	83	50
10/05	84	50
10/06	82	50
10/07	80	49
10/08	80	48
10/09	81	49
10/10	80	48
10/11	79	48
10/12	79	48
10/13	80	49
10/14	79	48
10/15	78	47
10/16	78	47
10/17	80	46
10/18	78	46
10/19	76	47
10/20	75	48
10/21	76	47
10/22	77	46
10/23	75	47
10/24	77	46
10/25	75	48
10/26	74	46
10/27	74	45
10/28	71	45
10/29	73	44
10/30	72	43
10/31	73	43

TABLE 9

Heat Tolerance Data for Study #4

#	ID	Heat Tolerance Rating*
1	4243-1	7.1
2	4263-1	7.2
3	4267-1	7.0
4		7.2
5	4274-1	6.9
6	4274-2	6.9
7	4278-1	7.4
8	4284-1	7.2
9	4285-1	6.8
10	4308-2	6.8
11	4309-1	6.7
12	4318-1	6.3
13	4320-1	7.0
14	4320-2	7.0
15	4321-1	7.1
16	4354-1	7.2
17	4354-2	7.2
18	4355-1	6.5
19	4377-1	7.1
20	4395-2	6.5
21	4412-1	6.9
22	4430-1	7.4
23	4432-1	6.9
24	4437-1	7.0
25	4450-1	6.6
26	4450-2	6.6
27	4460-1	7.1
28	4462-1	7.2
29	4465-1	7.3
30	4476-1	7.1
31	Pinnacle	5.0
32	Marathon	5.0
33	Greenbelt	4.5
34	7007	7.0
35	4201	7.0
36	4208	3.0
37	4209	6.0
38	4212	6.1
39	4219	7.0
40	4221	6.9

TABLE 9

Heat Tolerance Data for Study #4

#	ID	Heat Tolerance Rating*
41	4237	6.2
42	4280	6.1
43	4287	6.1
44	4288	7.3
45	4289	6.0
46	4290	6.7
47	4291/4459	7.2
48	4301	6.5
49	4303	7.1
50	4304	7.1
51	4317	6.4
52	4338	4.9
53	4370	6.2
54	4415	6.7
55	4418	5.5
56	4441	6.3
57	4442	6.3
58	4468	6.8
60	4470	6.5

* 0 = Most susceptible
5 = Intermediate
9 = Most tolerant

TABLE 10

Yield, Bead Size, Head Shape, Extension and Uniformity Data for Study #4

#	ID	Yield	Bead Size	Head Shape	Extension	Uniformity
1	4243-1	8.8	3.6	7.0	5.5	6.5
2	4263-1	7.0	3.4	7.3	7.3	6.8
3	4267-1	6.8	3.9	6.8	7.1	6.9
4	4267-1	6.9	3.9	7.1	7.2	
5	4274-1	7.2	3.5	7.3	7.5	6.8
6	4274-2	7.2	3.5	7.3	7.5	6.8
7	4278-1	7.6	3.7	7.4	7.2	6.8
8	4284-1	7.3	3.5	7.4	7.5	6.9
9	4285-1	7.4	3.7	7.3	7.3	6.0
10	4308-2	7.0	3.8	6.7	7.9	6.5
11	4309-1	6.9	3.7	6.5	8.2	6.4
12	4318-1		3.6	7.5	7.6	6.9
13	4320-1	7.0	3.6	7.5	7.5	7.0
14	4320-2	7.0	3.6	7.5	7.5	7.0
15	4321-1	7.0	3.7	7.6	7.2	6.9
16	4354-1	7.2	3.6	7.4	7.2	7.1
17	4354-2	7.2	3.6	7.4	7.2	7.1
18	4355-1	6.8	3.9	6.1	7.8	6.5
19	4377-1	7.0	3.7	7.4	7.0	6.8
20	4395-2	7.0	3.7	6.8	7.2	6.8
21	4412-1	7.0	3.8	6.5	7.8	6.1
22	4430-1	7.1	3.3	7.0	7.6	6.9
23	4432-1	7.4	3.5	7.2	7.5	7.0
24	4437-1	7.3	3.6	7.4	7.3	6.1

TABLE 10

Yield, Bead Size, Head Shape, Extension and Uniformity Data for Study #4

#	ID	Yield	Bead Size	Head Shape	Extension	Uniformity
25	4450-1	7.5	3.6	7.1	7.2	6.5
26	4450-2	7.5	3.6	7.1	7.2	6.5
27	4460-1	7.3	3.7	7.1	7.0	7.0
28	4462-1	7.2	3.5	7.3	7.3	6.7
29	4465-1	7.4	3.5	7.2	7.1	6.2
30	4476-1	7.8	3.5	7.4	7.1	6.5
31	Pinnacle	7.2	3.6	6.0	7.2	6.9
32	Marathon	7.3	3.3	6.0	7.0	6.7
33	Greenbelt	6.9	3.5	5.0	7.0	6.9
34	7007	7.5	3.8	7.3	7.1	7.0

TRANSGENIC BROCCOLI

The broccoli varieties of this invention can be transformed with useful genes to make heat tolerant transgenic broccoli varieties. Such useful genes include "terminator genes", herbicide resistant genes, insect resistant genes, virus resistant genes and the like.

To introduce isolated genes or a group of genes into the genome of plant cells such as broccoli an efficient host gene vector system is necessary. The foreign genes should be expressed in the transformed plant cells and consistently transmitted (somatically and sexually) to the next generation of cells produced. The vector should be capable of introducing, maintaining and expressing a gene in plant cells, from a variety of sources, including but not limited to plants and animals, bacteria, fungi, yeast or virus. Additionally it should be possible to introduce the vector into a wide variety of plants. The location of the new gene in the plant genome may be

important in determining effective gene expression of the genetically engineered plant. In addition, to be effective, the new gene must be passed on to progeny by normal breeding.

Directed genetic modification and expression of foreign genes in dicotyledonous (broad-leafed) plants such as tobacco, broccoli, potato and alfalfa has been shown to be possible using the T-DNA of the tumor-inducing (Ti) plasmid of Agrobacterium tumefaciens. Using recombinant DNA techniques and bacterial genetics, foreign pieces of DNA can be inserted into T-DNA in Agrobacterium. Following infection by the bacterium or Ti plasmid, the foreign DNA is inserted into the host plant chromosomes, thus producing a genetically engineered cell and eventually a genetically engineered plant. A second approach is to introduce root-inducing (Ri) plasmids as the gene vectors.

Transformation of broccoli is well known in the art of molecular biology. For example, in Cao, et al. "Transgenic broccoli with high levels of Bacillus thuringiensis CrylC protein control diamondback moth larvae resistant to CrylA or CrylC," Molecular Breeding 5:131-141 (1999), discloses one method of generation of transgenic broccoli. Such procedures can readily and easily be followed to produce transgenic heat tolerant broccoli plants.

ISOLATION OF HEAT TOLERANCE GENES

Now that heat tolerant broccoli plants have been identified and isolated, the identification of the gene or genes involved in heat tolerance is a straightforward process. One of ordinary skill in the art can identify genes involved in heat tolerance by comparing the DNA of heat tolerant and heat sensitive broccoli plants. One such method of isolating heat tolerance gene is the use of a matrix mill available from Cornell University in Ithaca, New York. The use of such a device greatly facilitates the isolation of heat tolerant genes. The device is capable of breaking up 96 small tissue samples simultaneously in sodium hydroxide, releasing the tissue's DNA and denaturing the protein. After the extraction, the tissue sample is then neutralized and the DNA is simultaneously diluted. Once diluted, the DNA is ready for analysis. Using the matrix mill one can compare several heat tolerant to several heat sensitive broccoli lines simultaneously.

In addition to using the matrix mill, basic molecular biological techniques may be utilized by one of ordinary skill in the art to isolate the heat tolerant broccoli gene. Such procedures are outlined in detail in Ausubel, et al. (Eds) (1987) "Current Protocols in Molecular Biology," John Wiley and Sons, New York.

Once the heat tolerant gene or genes are identified in broccoli, the corresponding heat tolerant gene or genes can be isolated in other plants through various hybridization techniques as described in Ausubel, et al.

Furthermore, biological material can be isolated from the seeds and plants of this invention by procedures well known in the art. Such material may include but is not limited to DNA, RNA, protein and carbohydrates.

DEPOSIT INFORMATION

Representative of, but not limiting the invention, Applicants have deposited seeds from M7028, M7007, M7009 and 393-2-19 with the American Type Culture Collection.

Applicants have made available to the public without restriction a deposit of at least 2500 seeds of broccoli M7028 with the American Type Culture Collection (ATCC), Rockville, MD 20852 which has been assigned ATCC number 203530.

Applicants have made available to the public without restriction a deposit of at least 2500 seeds of broccoli M7007 with the American Type Culture Collection (ATCC), Rockville, MD 20852 which has been assigned ATCC number 203531.

Applicants have made available to the public without restriction a deposit of at least 2500 seeds of broccoli M7009 with the American Type Culture Collection (ATCC), Rockville, MD 20852 which has been assigned ATCC number 203532.

Applicants have made available to the public without restriction a deposit of at least 2500 seeds of broccoli 393-2-19 with the American Type Culture Collection (ATCC), Rockville, MD 20852 which has been assigned ATCC number 203533.

The deposits will be maintained in the ATCC depository, which is a public depository, for a period of 30 years, or 5 years after the most recent request, or for the effective life of the patent, whichever is longer, and will be replaced if a deposit becomes nonviable during that period.

Although the foregoing invention has been described in some detail by way of illustration and examples for purposes of clarity and understanding, it will be obvious that certain modifications and alternative embodiments of the invention are contemplated which do not depart from the spirit and scope of the invention as defined by the foregoing teachings and appended claims.

We claim:

1. A heat tolerant broccoli plant.

2. Seed produced from the plant of claim 1.

3. Progeny seed produced from crossing the broccoli plant of claim 1 with another plant.

4. Broccoli plants produced from the seed of claim 3.

5. A broccoli seed capable of germinating into a plant which produces a commercially acceptable broccoli head under heat stress growth conditions.

6. The broccoli seed of claim 5 wherein said heat stress growth conditions are such that a broccoli variety selected from the group consisting of Marathon, Pinnacle, Premium Crop, Patriot, Laguna, Montecristo, Greenbelt, Sultan, Tierra, Laguna, Fiesta, Liberty and Landmark does not produce a commercially acceptable head.

7. The broccoli seed of claim 5 wherein said heat stress growth conditions include exposure of said plant to a maximum temperature of at least 90°F for at least 5 consecutive days during the growth cycle of said plant.

8. The broccoli seed of claim 5 wherein said heat stress growth conditions include exposure of said plant to a maximum temperature of at least 95°F for at least one day during the growth cycle of said plant.

9. The broccoli seed of claim 5 wherein said heat stress growth conditions include exposure of said plant to a maximum temperature of 85°F for at least 15 days during the growth cycle of said plant.

10. A broccoli plant or its parts produced by the seed of claim 5.

11. Broccoli plants regenerated from tissue culture of the broccoli plant of claim 10.

12. A seed from said plant of claim 10 or its progeny.

13. A broccoli seed designated 393-2-19 and having ATCC Accession Number 203533.

14. A broccoli plant or its parts produced by the seed of claim 13.

15. Broccoli plants regenerated from tissue culture of the broccoli plant of claim 14.

16. Progeny seed produced from crossing the plant of claim 14 with another plant.

17. Tissue culture according to claim 15 comprising regenerable cells selected from the group consisting of meristematic tissue, anthers, leaves, ovules, roots, embryos, protoplasts and pollen.

18. A broccoli plant regenerated from regenerable cells of a tissue culture according to claim 17.

19. A broccoli plant having all the phenotypic characteristics of a plant produced from the seed of claim 13.

20. A seed from said plant of claim 19 or its progeny.

21. A broccoli plant produced from the progeny seed of claim 16.

22. A broccoli seed produced from the broccoli plant of claim 20.

23. A broccoli seed designated M7028 and having ATCC Accession No. 203530.

24. A broccoli plant or its parts produced by the seed of claim 23.

25. Broccoli plants regenerated from tissue culture of the broccoli plant of claim 24.

26. Tissue culture according to claim 24 comprising regenerable cells selected from the group consisting of meristematic tissue, anthers, leaves, ovules, roots, embryos, protoplasts and pollen.

27. A broccoli plant regenerated from regenerable cells of a tissue culture according to claim 26.

28. A broccoli plant having all the phenotypic characteristics of a plant produced from the seed of claim 23.

29. A seed from said plant of claim 24 or its progeny.

30. Progeny seed produced from crossing the plant of claim 24 with another broccoli plant.

31. A broccoli plant produced from the progeny seed of claim 30.

32. A broccoli seed produced from the broccoli plant of claim 31.

33. A broccoli seed designated M7007 and having ATCC Accession No. 203531.

34. A broccoli plant or its parts produced by the seed of claim 33.

35. Broccoli plants regenerated from tissue culture of the broccoli plant of claim 34.

36. Tissue culture according to claim 35 comprising regenerable cells selected from the group consisting of meristematic tissue, anthers, leaves, ovules, roots, embryos, protoplasts and pollen.

37. A broccoli plant having all the phenotypic characteristics of a plant produced from the seed of claim 33.

38. A seed from said plant of claim 34 or its progeny.

39. Progeny seed produced from crossing the plant of claim 34 with another plant.

40. A broccoli plant produced from the progeny seed of claim 39.

41. A broccoli seed designated M7009 and having ATCC Accession No. 203532.

42. A broccoli plant or its parts produced by the seed of claim 41.

43. Broccoli plants regenerated from tissue culture of the broccoli plant of claim 42.

44. Tissue culture according to claim 43 comprising regenerable cells selected from the group consisting of meristematic tissue, anthers, leaves, ovules, roots, embryos, protoplasts and pollen.

45. A broccoli plant having all the phenotypic characteristics of a plant produced from the seed of claim 41.

46. A seed from said plant of claim 42 or its progeny.

47. Progeny seed produced from crossing the plant of claim 42 with another plant.

48. A broccoli plant produced from the progeny seed of claim 47.

49. A broccoli seed selected from the group consisting of those broccoli seeds designated H7008, H7022, 393-2-47, 98-2192, 98-2088, 98-2061, H7007 and H70028.

50. A broccoli plant or its parts produced by the seed of claim 49.

51. A seed from said plant of claim 50 or its progeny.

52. A seed produced from crossing the plant of claim 50 with another broccoli plant.

53. A broccoli seed selected from the group consisting of those broccoli seeds designated: M7028, M7007, M7009, M7022, 393-2-19, H7008, H7022, 393-2-47, 98-2192, 98-2088, 98-2061, H7007, H7028, H7010, and H7021R, 4243, 4221, 4441, 4274-1, 4274-2, 4278-1, 4284-1, 4285-1, 4354-1, 4354-2, 4377-1, 4318-1, 4320-1, 4320-2, 4321-1, 4437-1, 4476-1, 4462-1, 4308-2, 4309-1, 4355-1, 4412-1, 4301, 4303, 4304, 4317, 4468, 4470, 4471, 4263-1, 4430-1, 4450-1, 4450-2, 4432-1, 4267-1, 7861, 7864, 7865, 7881, 7887, 7935, 8092, 7883, 7914, 7770, 7778, 4201, 4219, 4237, 4280, 4287, 4288, 4289, 4290, 4291, 4458-1, 4460-1, 4415, 4418, 4395-2.

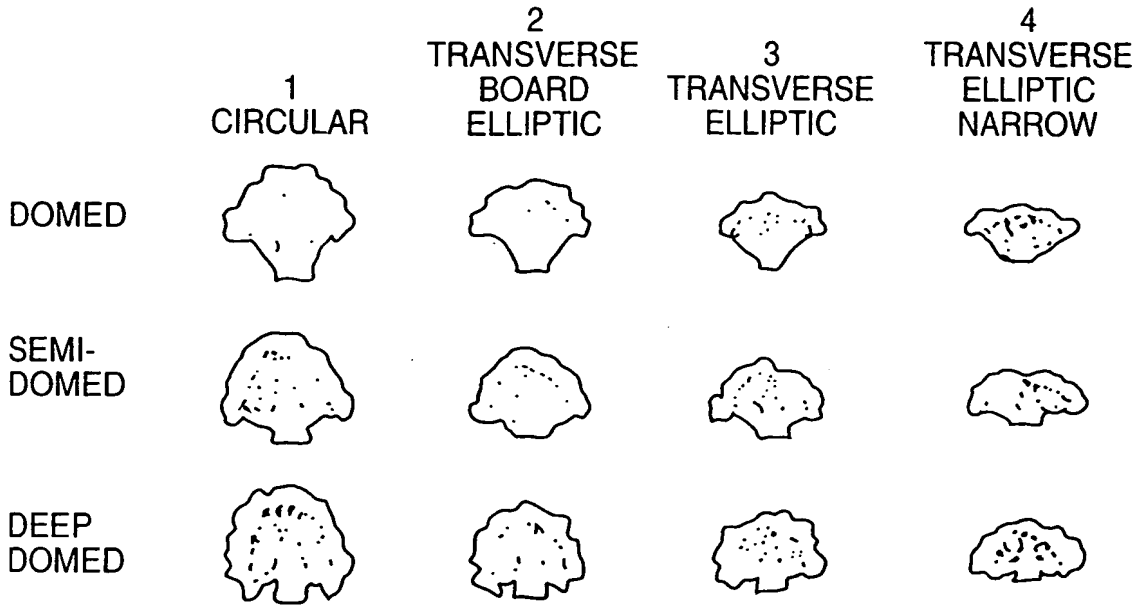

1
CIRCULAR
2
TRANSVERSE
BOARD
ELLIPTIC
3
TRANSVERSE
ELLIPTIC
4
TRANSVERSE
ELLIPTIC
NARROW
DOMED
SEMI-
DOMED
DEEP
DOMED

FIG. 1

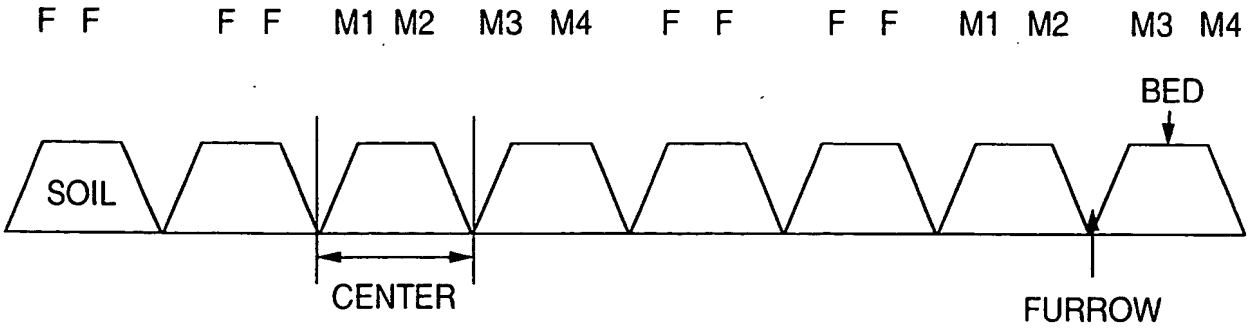

F F
F F
M1 M2
M3 M4
F F
F F
M1 M2
M3 M4
BED
SOIL
CENTER
FURROW

FIG. 2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/31230

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A01H 5/00, 5/02, 5/04, 5/06, 5/08, 5/10, 5/12, 4/00; C12N 5/04
US CL :800/306, 298, 260, 278; 435/410, 430

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 800/306, 298, 260, 278; 435/410, 430

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

RHS Dictionary of Gardening

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE, AGRICOLA, WEST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X - Y	YANG et al. A Heat-tolerant Broccoli R1 Hybrid, 'Ching-Long 45'. HortScience. 1998, Vol. 33, No. 6, pages 1090-1091, see entire document.	1-5 ---- 6-53
X - Y	HEATHER et al. Heat Tolerance and Holding Ability in Broccoli. Journal of the American Society for Horticultural Science. 1992, Vol. 117, No. 6, pages 887-892, see entire document.	1-5 ---- 6-53
X - Y	DUFAULT, R.J. Dynamic Relationships Between Field Temperatures and Broccoli Head Quality. Journal of the American Society for Horticultural Science. 1996, Vol. 121, No. 4, pages 705-710, see especially page 709, col. 2.	1-5 ---- 6-53

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
21 MARCH 2000

Date of mailing of the international search report
26 APR 2000

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
MELISSA KIMBALL
Telephone No. (703) 308-0196

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/31230

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SULLIVAN et al. Plant Responses to High Temperatures, Chapter 28, Genetic Diversity in Plants. Plenum Press, New York and London. 1977, pages 301-317, see entire document.	1-53

Corrected by IPEA/US

From the INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PTO/PCT 28 JUN 2001

JUL 24 2000

PCT LIMBACH & LIMBACH LLP

To:

MICHAEL R. WARD
LIMBACH & LIMBACH L.L.P.
2001 FERRY BUILDING
SAN FRANCISCO CA 94111-4262

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NOTIFICATION OF RECEIPT OF DEMAND BY COMPETENT INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

(PCT Rule 59.3(e) and 61.1(b), first sentence and Administrative Instructions, Section 601(a))

Applicant's or agent's file reference: RDAG-120 PCT	Date of mailing (day/month/year): 18 JUL 2000
International application No.: PCT/US99/31230	International filing date (day/month/year): 29 DEC 99
Priority date (day/month/year): 29 DEC 98	
Applicant: R&D AG INC.	

IMPORTANT NOTIFICATION

1. The applicant is hereby notified that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

02 JUN 2000 (02.06.00)

2. That date of receipt is:

- [X] the actual date of receipt of the demand by this Authority (Rule 61.1(b)).
- [ ] the actual date of receipt of the demand on behalf of this Authority (Rule 59.3(e)).
- [ ] the date on which this Authority has, in response to the invitation to correct defects in the demand (Form PCT/IPEA/404), received the required corrections.

3. [ ] **ATTENTION:** That date of receipt is **AFTER** the expiration of 19 months from the priority date. Consequently, the election(s) made in the demand does (do) not have the effect of postponing the entry into the national phase until 30 months from the priority date (or later in some Offices) (Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22). For details, see the *PCT Applicant's Guide*, Volume II.

[ ] *(If applicable)* This notification confirms the information given by telephone, facsimile transmission or in person on: _____

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/US Assistant Commissioner for Patents Box PCT Washington, D.C. 20231 Attn: IPEA/US Facsimile No.	Authorized officer Darlene Proctor dP PCT Operations - IAPD Team I Telephone No. 305-3389 (703) 305-3230 FAX

Form PCT/IPEA/402 (July 1998)

TENT COOPERATION TREA[illegible]

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: MICHAEL R. WARD
LIMBACH & LIMBACH L.L.P.
2001 FERRY BUILDING
SAN FRANCISCO, CA 94111-4207

RECEIVED
OCT 23 2000
Limbach & Limbach

PCT

WRITTEN OPINION

(PCT Rule 66)

Date of Mailing *(day/month/year)*	19 OCT 2000
Applicant's or agent's file reference: RDAG-120 PCT	REPLY DUE within TWO months from the above date of mailing

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/31230	29 DECEMBER 1999	29 DECEMBER 1998

International Patent Classification (IPC) or both national classification and IPC
Please See Supplemental Sheet.

Applicant
R&D AG INC.

Resp. Due 12/19/00

1. This written opinion is the first (first, etc.) drawn by this International Preliminary Examining Authority.

2. This opinion contains indications relating to the following items:

I	[X]	Basis of the opinion
II	[ ]	Priority
III	[ ]	Non-establishment of opinion with regard to novelty, inventive step or industrial applicability
IV	[ ]	Lack of unity of invention
V	[X]	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	[ ]	Certain documents cited
VII	[ ]	Certain defects in the international application
VIII	[ ]	Certain observations on the international application

3. The applicant is hereby invited to reply to this opinion.

When? See the time limit indicated above. ~~The applicant may, before the expiration of that time limit, request this Authority to grant an extension., see Rule 66.2(d).~~

How? By submitting a written reply, accompanied, where appropriate, by amendments, according to Rule 66.3. For the form and the language of the amendments, see Rules 66.8 and 66.9.

Also For an additional opportunity to submit amendments, see Rule 66.4.
For the examiner's obligation to consider amendments and/or arguments, see Rule 66.4 *bis*.
For an informal communication with the examiner, see Rule 66.6.

If no reply is filed, the international preliminary examination report will be established on the basis of this opinion.

4. The final date by which the international preliminary examination report must be established according to Rule 69.2 is: 29 APRIL 2001

Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer Dorothea Laurence for MELISSA KIMBALL
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/IPEA/408 (cover sheet) (July 1998)*

Resp. to Written Opin. Due
DOCKETED SV
12/6/00
12/19/00E

WRITTEN OPINION

International application No.
PCT/US99/31230

I. Basis of the opinion

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed

[X] the description:
pages 1-102 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the claims:
pages 103-108 , as originally filed
pages NONE , as amended (together with any statement) under Article 19
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the drawings:
pages 1 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the sequence listing part of the description:
pages NONE , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the written opinion was drawn on the basis of the sequence listing:

[ ] contained in the international application in printed form.

[ ] filed together with the international application in computer-readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages NONE

[X] the claims, Nos. NONE

[X] the drawings, sheets/~~fig~~ NONE

5. [ ] This opinion has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this opinion as "originally filed".*

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

TIME LIMIT:

The time limit set for response to a Written Opinion may not be extended. 37 CFR 1.484(d). Any response received after the expiration of the time limit set in the Written Opinion will not be considered in preparing the International Preliminary Examination Report.

CLASSIFICATION:

The International Patent Classification (IPC) and/or the National classification are as listed below:
IPC(7): A01H 5/00, 5/02, 5/04, 5/06, 5/08, 5/10, 5/12, 4/00; C12N 5/04 and US Cl.: 800/306, 298, 260, 278; 435/410, 430

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):
and seasons acceptable for production of this crop.

---------- NEW CITATIONS ----------

NONE

WRITTEN OPINION

International application No.
PCT/US99/31230

V. Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	9, 11, 13-53	YES
	Claims	1-8, 10, 12	NO
Inventive Step (IS)	Claims	13-53	YES
	Claims	1-12	NO
Industrial Applicability (IA)	Claims	1-53	YES
	Claims	NONE	NO

2. citations and explanations

Claims 1-8, 10 and 12 lack novelty under PCT Article 33(2) as being anticipated by Heather et al.

Heather et al. teach a broccoli cultivar 'XPH 5168' which is heat tolerant. It produces market acceptable broccoli heads at 35 degrees Celsius (95 degrees fahrenheit), (Table 4 and 5).

Claims 1-12 lack an inventive step under PCT Article 33(3) as being obvious over Heather et al. in view of Dufault.

Heather et al. teach heat tolerant broccoli which can tolerate a heat treatment of one week at 95 degrees fahrenheit (page 891, col. 1).

Heather et al. do not teach tolerance to a 15 day heat treatment of 85 degrees fahrenheit nor do they teach a method of tissue culture production for broccoli.

Dufault teaches that heat adversely affects floral development in broccoli and that heat tolerant broccoli would be useful for production of this crop during summer months in southeastern states (page 705, col. 1). Dufault teaches that 'Baccus' has acceptable color, bract number and compactness when grown at 32 degrees celsius (89.6 degrees fahrenheit) (page 708-709).

A skilled plant breeder would recognize the need to produce heat tolerant broccoli cultivars such as those taught by Heather et al. which can withstand even longer heat treatments because production of this crop in hot southeastern climates is desirable, as taught by Dufault. A plant breeder would want to reproduce such a heat tolerant plant by tissue culture to ensure that the trait is expressed in clonal offspring.

Claims 13-53 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest these specific broccoli cultivars.

Claims 1-53 meet the criteria set out in PCT Article 33(4), because heat tolerant broccoli cultivars would expand the regions

(Continued on Supplemental Sheet.)

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF RECEIPT OF RECORD COPY

(PCT Rule 24.2(a))

RECEIVED
MAR 2 0 2000
LIMBACH & LIMBACH L.L.P.

To:

WARD, Michael, R. EAR
Limbach & Limbach L.L.P.
2001 Ferry Building
San Francisco, CA 94111-4262
ÉTATS-UNIS D'AMÉRIQUE

Date of mailing (day/month/year)	IMPORTANT NOTIFICATION
03 March 2000 (03.03.00)	
Applicant's or agent's file reference	**International application No.**
RDAG-120 PCT	PCT/US99/31230

The applicant is hereby notified that the International Bureau has received the record copy of the international application as detailed below.

Name(s) of the applicant(s) and State(s) for which they are applicants:

R&D AG INC. (for all designated States except US)
BARHAM, Robert et al (for US)

International filing date	:	29 December 1999 (29.12.99)
Priority date(s) claimed	:	29 December 1998 (29.12.98) 08 June 1999 (08.06.99)
Date of receipt of the record copy by the International Bureau	:	18 February 2000 (18.02.00)
List of designated Offices	:	

AP :GH,GM,KE,LS,MW,SD,SL,SZ,TZ,UG,ZW
EA :AM,AZ,BY,KG,KZ,MD,RU,TJ,TM
EP :AT,BE,CH,CY,DE,DK,ES,FI,FR,GB,GR,IE,IT,LU,MC,NL,PT,SE
OA :BF,BJ,CF,CG,CI,CM,GA,GN,GW,ML,MR,NE,SN,TD,TG
National :AE,AL,AM,AT,AU,AZ,BA,BB,BG,BR,BY,CA,CH,CN,CR,CU,CZ,DE,DK,DM,EE,ES,FI,GB,GD,GE,GH,GM,HR,HU,ID,IL,IN,IS,JP,KE,KG,KP,KR,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,NO,NZ,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,US,UZ,VN,YU,ZA,ZW

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer:
J. Leitao

Telephone No. (41-22) 338.83.38

Continuation of Form PCT/IB/30.

NOTIFICATION OF RECEIPT OF RECORD COPY

Date of mailing (day/month/year)	IMPORTANT NOTIFICATION
03 March 2000 (03.03.00)	
Applicant's or agent's file reference	**International application No.**
RDAG-120 PCT	PCT/US99/31230

ATTENTION

The applicant should carefully check the data appearing in this Notification. In case of any discrepancy between these data and the indications in the international application, the applicant should immediately inform the International Bureau.

In addition, the applicant's attention is drawn to the information contained in the Annex, relating to:

- [X] time limits for entry into the national phase
- [ ] confirmation of precautionary designations
- [X] requirements regarding priority documents

A copy of this Notification is being sent to the receiving Office and to the International Searching Authority.

INFORMATION ON TIME LIMITS FOR ENTERING THE NATIONAL PHASE

The applicant is reminded that the "national phase" must be entered before each of the designated Offices indicated in the Notification of Receipt of Record Copy (Form PCT/IB/301) by paying national fees and furnishing translations, as prescribed by the applicable national laws.

The time limit for performing these procedural acts is **20 MONTHS** from the priority date or, for those designated States which the applicant elects in a demand for international preliminary examination or in a later election, **30 MONTHS** from the priority date, provided that the election is made before the expiration of 19 months from the priority date. Some designated (or elected) Offices have fixed time limits which expire even later than 20 or 30 months from the priority date. In other Offices an extension of time or grace period, in some cases upon payment of an additional fee, is available.

In addition to these procedural acts, the applicant may also have to comply with other special requirements applicable in certain Offices. **It is the applicant's responsibility** to ensure that the necessary steps to enter the national phase are taken in a timely fashion. Most designated Offices do not issue reminders to applicants in connection with the entry into the national phase.

For detailed information about the procedural acts to be performed to enter the national phase before each designated Office, the applicable time limits and possible extensions of time or grace periods, and any other requirements, see the relevant Chapters of Volume II of the PCT Applicant's Guide. Information about the requirements for filing a demand for international preliminary examination is set out in Chapter IX of Volume I of the PCT Applicant's Guide.

GR and ES became bound by PCT Chapter II on 7 September 1996 and 6 September 1997, respectively, and may, therefore, be elected in a demand or a later election filed on or after 7 September 1996 and 6 September 1997, respectively, regardless of the filing date of the international application. (See second paragraph above.)

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

CONFIRMATION OF PRECAUTIONARY DESIGNATIONS

This notification lists only specific designations made under Rule 4.9(a) in the request. It is important to check that these designations are correct. Errors in designations can be corrected where precautionary designations have been made under Rule 4.9(b). The applicant is hereby reminded that any precautionary designations may be confirmed according to Rule 4.9(c) before the expiration of 15 months from the priority date. If it is not confirmed, it will automatically be regarded as withdrawn by the applicant. There will be no reminder and no invitation. Confirmation of a designation consists of the filing of a notice specifying the designated State concerned (with an indication of the kind of protection or treatment desired) and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.

REQUIREMENTS REGARDING PRIORITY DOCUMENTS

For applicants who have not yet complied with the requirements regarding priority documents, the following is recalled.

Where the priority of an earlier national, regional or international application is claimed, the applicant must submit a copy of the said earlier application, certified by the authority with which it was filed ("the priority document") to the receiving Office (which will transmit it to the International Bureau) or directly to the International Bureau, before the expiration of 16 months from the priority date, provided that any such priority document may still be submitted to the International Bureau before that date of international publication of the international application, in which case that document will be considered to have been received by the International Bureau on the last day of the 16-month time limit (Rule 17.1(a)).

Where the priority document is issued by the receiving Office, the applicant may, instead of submitting the priority document, request the receiving Office to prepare and transmit the priority document to the International Bureau. Such request must be made before the expiration of the 16-month time limit and may be subjected by the receiving Office to the payment of a fee (Rule 17.1(b)).

If the priority document concerned is not submitted to the International Bureau or if the request to the receiving Office to prepare and transmit the priority document has not been made (and the corresponding fee, if any, paid) within the applicable time limit indicated under the preceding paragraphs, any designated State may disregard the priority claim, provided that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity to furnish the priority document within a time limit which is reasonable under the circumstances.

Where several priorities are claimed, the priority date to be considered for the purposes of computing the 16-month time limit is the filing date of the earliest application whose priority is claimed.

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

To:

WARD, Michael, R.
Limbach & Limbach L.L.P.
2001 Ferry Building
San Francisco, CA 94111-4262
ETATS-UNIS D'AMERIQUE

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

Date of mailing (day/month/year) 23 March 2000 (23.03.00)	
Applicant's or agent's file reference RDAG-120 PCT	**IMPORTANT NOTIFICATION**
International application No. PCT/US99/31230	International filing date (day/month/year) 29 December 1999 (29.12.99)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 29 December 1998 (29.12.98)
Applicant R&D AG INC. et al	20 mo date 8/29/00 30 mo date 6/29/01 on del

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.

3. An **asterisk(*)** appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

4. The **letters "NR"** appearing in the right-hand column denote a priority document which was not received by the International Bureau or which the applicant did not request the receiving Office to prepare and transmit to the International Bureau, as provided by Rule 17.1(a) or (b), respectively. In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
29 Dece 1998 (29.12.98)	60/114,038	US	14 Marc 2000 (14.03.00)
08 June 1999 (08.06.99)	09/328,121	US	14 Marc 2000 (14.03.00)

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer

Taïeb Akremi

Telephone No. (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

To:

WARD, Michael, R.
Morrison & Foerster, LLP
755 Page Mill Road
Palo Alto, CA 94304-1018
ETATS-UNIS D'AMERIQUE

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

Date of mailing (day/month/year) 11 January 2001 (11.01.01)	
Applicant's or agent's file reference 500852000140	**IMPORTANT NOTIFICATION**
International application No. PCT/US99/31230	**International filing date** (day/month/year) 29 December 1999 (29.12.99)

1. The following indications appeared on record concerning:

[ ] the applicant [ ] the inventor [X] the agent [ ] the common representative

Name and Address	State of Nationality	State of Residence
WARD, Michael, R. Limbach & Limbach L.L.P. 2001 Ferry Building San Francisco, CA 94111-4262 United States of America		

Telephone No.: (415) 433-4150
Facsimile No.: (415) 433-8716
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[ ] the person [ ] the name [X] the address [ ] the nationality [ ] the residence

Name and Address	State of Nationality	State of Residence
WARD, Michael, R. Morrison & Foerster, LLP 755 Page Mill Road Palo Alto, CA 94304-1018 United States of America		

Telephone No.: (650) 813-5600
Facsimile No.: (650) 494-0792
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland** Facsimile No.: (41-22) 740.14.35	Authorized officer J. Leitao Telephone No.: (41-22) 338.83.38

WO 00/38500
PCT/US99/3123(

PATENT COOPERATION TREATY

PCT

From the INTERNATIONAL BUREAU

To:
WARD, Michael, R.
Limbach & Limbach L.L.P.
2001 Ferry Building
San Francisco, CA 94111-4262
ETATS-UNIS D'AMERIQUE

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

Date of mailing (day/month/year) 06 July 2000 (06.07.00)	
Applicant's or agent's file reference RDAG-120 PCT	**IMPORTANT NOTICE**

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/US99/31230	29 December 1999 (29.12.99)	29 December 1998 (29.12.98)

Applicant
R & D AG INC. et al

20 mo date 8/29/00
30 mo date 6/29/01 or rd

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AU,CN,JP,KP,KR,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   AE,AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CR,CU,CZ,DE,DK,DM,EA,EE,EP,ES,FI,GB,GD,GE,GH,GM,HR,HU,ID,IL,IN,IS,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,NO,NZ,OA,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW
   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   06 July 2000 (06.07.00) under No. WO 00/38500

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer
J. Zahra

Telephone No. (41-22) 338.83.38

ATENT COOPERATION TRE[redacted]Y

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: MICHAEL R. WARD
LIMBACH & LIMBACH L.L.P.
2001 FERRY BUILDING
SAN FRANCISCO, CA 94111-4207

RECEIVED BY
PCT 0 2 2001
MORRISON & FOERSTER

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of Mailing *(day/month/year)*	18 APR 2001
Applicant's or agent's file reference: RDAG-120 PCT	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority Date *(day/month/year)*
PCT/US99/31230	29 DECEMBER 1999	29 DECEMBER 1998

Applicant
R&D AG INC.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

DOCKETED GAP 3cc

Name and mailing address of the IPEA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	MELISSA KIMBALL Kathrea Lawrence For
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/IPEA/416 (July 1992)★

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
RDAG-120 PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/31230	29 DECEMBER 1999	29 DECEMBER 1998
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant R&D AG INC.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report

   II ☐ Priority

   III ☐ Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV ☐ Lack of unity of invention

   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI ☐ Certain documents cited

   VII ☐ Certain defects in the international application

   VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
02 JUNE 2001	23 MARCH 2001
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Dorothea Laurence for MELISSA KIMBALL Telephone No. (703) 308-0196

Form PCT/IPEA/409 (cover sheet) (July 1998)★

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US99/31230

I. Basis of the report

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed

[X] the description:
pages 1-102 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the claims:
pages 103-108 , as originally filed
pages NONE , as amended (together with any statement) under Article 19
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the drawings:
pages 1 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the sequence listing part of the description:
pages NONE , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained in the international application in printed form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages NONE
[X] the claims, Nos. NONE
[X] the drawings, sheets/~~fig~~ NONE

5. [ ] This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US99/31230

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	9, 11, 13-53	YES
	Claims	1-8, 10, 12	NO
Inventive Step (IS)	Claims	13-53	YES
	Claims	1-12	NO
Industrial Applicability (IA)	Claims	1-53	YES
	Claims	NONE	NO

2. citations and explanations (Rule 70.7)

Claims 1-8, 10 and 12 lack novelty under PCT Article 33(2) as being anticipated by Heather et al.

Heather et al. teach a broccoli cultivar 'XPH 5168' which is heat tolerant. It produces market acceptable broccoli heads at 35 degrees Celsius (95 degrees fahrenheit), (Table 4 and 5).

Claims 1-12 lack an inventive step under PCT Article 33(3) as being obvious over Heather et al. in view of Dufault.

Heather et al. teach heat tolerant broccoli which can tolerate a heat treatment of one week at 95 degrees fahrenheit (page 891, col. 1).

Heather et al. do not teach tolerance to a 15 day heat treatment of 85 degrees fahrenheit nor do they teach a method of tissue culture production for broccoli.

Dufault teaches that heat adversely affects floral development in broccoli and that heat tolerant broccoli would be useful for production of this crop during summer months in southeastern states (page 705, col. 1). Dufault teaches that 'Baccus' has acceptable color, bract number and compactness when grown at 32 degrees celsius (89.6 degrees fahrenheit) (page 708-709).

A skilled plant breeder would recognize the need to produce heat tolerant broccoli cultivars such as those taught by Heather et al. which can withstand even longer heat treatments because production of this crop in hot southeastern climates is desirable, as taught by Dufault. A plant breeder would want to reproduce such a heat tolerant plant by tissue culture to ensure that the trait is expressed in clonal offspring.

Claims 13-53 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest these specific broccoli cultivars.

Claims 1-53 meet the criteria set out in PCT Article 33(4), because heat tolerant broccoli cultivars would expand the regions

(Continued on Supplemental Sheet.)

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

CLASSIFICATION:
The International Patent Classification (IPC) and/or the National classification are as listed below:
IPC(7): A01H 5/00, 5/02, 5/04, 5/06, 5/08, 5/10, 5/12, 4/00; C12N 5/04 and US Cl.: 800/306, 298, 260, 278; 435/410, 430

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):
and seasons acceptable for production of this crop.

---------- NEW CITATIONS ----------
NONE